CALCULATING ANCESTRAL INFLUENCE IN MAN: A MATHEMATICAL MEASURE OF THE FACTS OF BISEXUAL HEREDITY[1]

HARRY H. LAUGHLIN

Eugenics Record Office, Cold Spring Harbor, Long Island, New York

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According to the common method of calculating ancestral influence in man, the germ-plasm is considered as an indefinitely dilutable substance contributed equally by the father and mother in making up the hereditary constitution of the offspring. Barring special phenomena, the several direct ancestors in each generation are, among them, quite properly accredited with the possession, patently or latently, of all of the traits of a particular offspring. In calculating the influence of a given ancestor in contributing toward the whole body of these traits in the offspring, the measure is a quotient determined by dividing unity by the number of direct ancestors in the given generation. Thus, the ancestral influence of one of the four grandparents is commonly said to be one-fourth, that of one of the eight great-grandparents one-eighth, and so on.

Sir FRANCIS GALTON, after a very careful study of the inheritance of human stature, proposed a modification of this orthodox notion of ancestral influence. His scheme is more in consonance with many of the observed facts than is the common method of dividing unity by the number of direct ancestors in the particular generation. His rule, or GALTON'S Law as it is sometimes called, ascribes one-half of all ancestral influence to the two parents (each parent one-half of 50 percent, or 25 percent), one-fourth to the four grandparents (each grandparent one-fourth of 25 percent, or 6.25 percent), one-eighth to the eight great-grandparents (each great-grandparent one-eighth of 12.5 percent, or 1.5625 percent). Later KARL PEARSON made some mathematical refinements of this scheme. But the study of pedigrees is so replete with cases showing the segregation and re-combination of ancestral traits that, even when traits are massed and averaged, neither the common quotients of the genealogists nor the generalizations of GALTON or PEARSON suffice to explain the pedigree-facts, nor does either scheme square with the cytological evidence in the case.

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With the rise of Mendelian investigations and the discovery of the close co-ordination between Mendelian pedigree-findings and the cytological evidence of the mechanism of heredity, there was founded the modern science of heredity, which is much more generally consistent with the observed facts than are any of the older systems of gauging natural inheritance.

By ancestral influence is meant the contribution of definite hereditary mental, physical and moral qualities, by a given ancestor to a given descendant. Pedigree-studies have set forth in man, as in many other organisms, rules of inheritance of many specific traits or qualities. Cytological investigations have shown how chromosomes duplicate, segregate and recombine (sometimes crossing-over and showing other special phenomena) in the course of gametogenesis, fertilization and development.

Neither the human nor any other germ-plasm is indefinitely dilutable or divisible, as many genealogists have assumed, but the dilutability and divisibility of the germ-plasm are limited by the number of chromosomes characteristic of the gametes of the particular species. In the human zygote two gametes, each with 12 chromosomes, unite.[2] This means that, barring crossing-over, mutation, and other special phenomena, normally the human germ-plasm, and consequently all hereditary human qualities, that is, the "blood," is divisible only into 24 uneven units corresponding to the 24 zygotic chromosomes.

It is well demonstrated that the chromosomes are composed of genes or the determiners of natural inheritance. Each chromosome is a generadical or linkage-group of trait determiners. If then it is sought to measure the influence of a given ancestor upon the hereditary traits of a given propositus, it is necessary to develop a series of mathematical formulae which measure the probabilities of definite situations happening in reference to the transmission of chromosomes from the given ancestor to the given propositus.

In developing such formulae the following factors must be taken into consideration:

First. Whether the species in question is bisexual.

[2] There is still disagreement among cytologists concerning the number of chromosomes in man. VON WINIWARTER (1912) concludes that the zygotic number of chromosomes in the human male is 47, in the human female 48, while WEIMAN (1917) concludes that the zygotic or diploid number of chromosomes is 24, of which 2 are the XY idiochromosomes. When this problem is settled beyond peradventure, the correct number can be used in the accompanying formulae, which are here stated in general terms. For a history of the cytological study of the chromosome number in man see the excellent analytical bibliography given by ETHEL BROWNE HARVEY in Journal of Morphology 34: 47–49, June, 1920.

Second. Whether in the particular species the male or the female is the heterozygous sex.

Third. Due provision must be made for sex-linked traits.

Fourth. The number of chromosomes, both gametic and zygotic, characteristic of the species, must be known.

Fifth. For the present the individual chromosomes will not be weighted, but simply counted. Doubtless the ontogenetic working out of each chromosome is very specific both qualitatively and quantitatively. When ultimately genetical and cytological research locate in definite chromosomes the genes for definite traits, then the several chromosomes can be weighted, and due provision for their differential values properly made in the formulae for measuring the several features of ancestral influence.

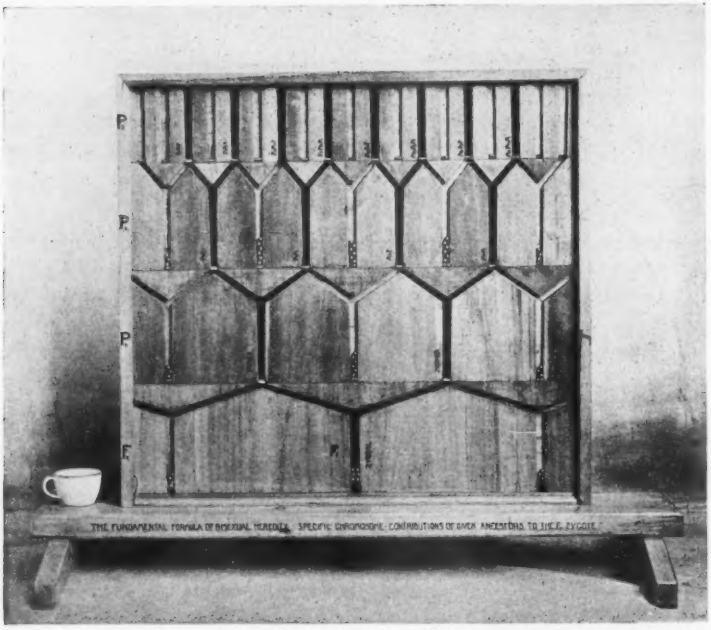
Sixth. Due cognizance must be taken of the possibility of mutation, crossing-over, non-disjunction and other special chromosomal phenomena occurring in the course of the descent of chromosomes from a given ancestor to the propositus. In the accompanying formulae such phenomena are not provided for, because their rates of occurrence are not yet measurable in man, but when one by one their mathematical measures are attained, each such measure can be woven into the foundation formulae here given. The present formulae will not be upset by such corrections, but simply modified in the direction of greater accuracy.

Seventh. It must be considered that in a given gamete a definite chromosome may have been derived from either the paternal or the maternal source. This is especially important in cases of zygotes of mixed or mongrel blood.

Eighth. In certain situations it will be necessary to specify whether a given trait is dominant or recessive to its allelomorph.

Finally, all formulae must be stated in general terms, so that they may apply with equal accuracy to any species of plant or animal concerning which chromosomal data are at hand.

Applying these principles to man, the accompanying charts set forth in pedigree-fashion and measure mathematically a few selected definite features of the hereditary influence of each particular ancestor taken as a type, upon the propositus or F_1 zygote. These calculations are based upon the following data and conditions: (a) that the gametic number of human chomosomes is 12, the zygotic or somatic number in each sex 24; (b) that the male is heterozygous (XY) and the female homozygous (XX) in reference to sex; (c) that all of the purely hereditary qualities are carried by the several chromosomes; and (d) that in the first or foundation formulae no provision will be made for mutation, crossing-over, non-disjunction and other special phenomena.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THE FUNDAMENTAL FORMULA OF BISEXUAL HEREDITY. SPECIFIC CHROMOSOME CONTRIBUTIONS OF EACH ANCESTOR TO THE F1 ZYGOTE

FIGURE 1.—Mechanism showing the essential features of segregation and recombination in the transmission of chromosomes from ancestors to descendants.

In the case here pictured, as shown by the location of the balls, the descent of chromosomes is traced in a bisexual species characterized by three pairs of chromosomes. Into each of the eight slots representing the eight ancestors of the P_3 generation, six balls were fed (through openings in the top of the machine not shown in the figure), to represent the six somatic chromosomes (three pairs). If the balls are given a distinct color or stamp for each of these eight ancestors, the tracing of the individual balls becomes clearer. The slots are constructed so that the balls, when fed into them, will settle alternately to the right and left, or *vice versa*, depending upon the chance settling of the first ball. The dividing wedge below the slot is located with its apex on the mid-line of the slot, so that when the slide is withdrawn from the back the balls pass alternately to the right and left, or *vice versa*, depending upon the particular system of stacking. Thus in the operation of the machine the segregation and recombination of balls follows the same mathematical rules which (barring crossing over, non-disjunction, mutation, special systems of selection, and other special phenomena) govern the descent of chromosomes in normal bisexual heredity. The fundamental formulae given in the accompanying paper may be demonstrated by its use.

This machine is 33 inches by $35\frac{1}{2}$ inches in size, not including the base. Its face is covered with glass $\frac{3}{16}$ inch thick. The dark line traces ancestral descent for the F_1 zygote. At the bottom of each slot there is a wooden stop which withdraws from the back, and below each *cul-de-sac* slot there is a tin chute which opens to the rear, and enables the operator conveniently to remove the balls. The three lowest slots also open into similar chutes.

Assuming the above conditions (and assuming that, until future research results in weighting ontogenetic values of specific chromosomes, each chromosome carries about the same number of genes or determiners for traits as each of the other 11 chromosomes), we find that we should speak of "blood" or ancestral influence not alone in terms of quotients obtained by dividing unity by the number of direct ancestors of the particular generation, although this has a certain genealogical value, but should measure ancestral influence by the probability that a given number of chromosomes or gene-radicals, according to the demonstrated laws of heredity, will descend from the given ancestors to the particular propositus.

Under the old scheme, each one of the 16 great-great-grandparents was thought of as contributing one-sixteenth of the "blood" possessed by the descendants of the F_1 generation. The average contribution in chromosome-number, (barring the sex-chromosome, which follows much more restricted paths in the family-tree), characteristic of such an ancestor, is 1.375. This, of course, is one-sixteenth of the eleven equivalent chromosome-pairs. But this is not a constant contribution. The range of chromosome-contribution by members of the great-great-grandparental generation, barring those individuals in the X- or Y-trails, varies from none to eleven chromosomes. Each particular case must, of course, be analyzed by pedigree-methods to determine what the actual contribution has been in the case of an individual already born. Actually, (barring special phenomena), each parent or ancestor contributes either none or a definite unit number of chromosomes. This number may be as high as 12 in man, or in any species as high as the gametic number of chromosomes. A fractional number following formula A on the pedigree-charts (plates 1 and 2) measures an average calculated in reference to the person, taken as a type, occupying the given position in the pedigree.

It is evident that if we wish a more accurate measure of ancestral influence than is given by the ordinary methods, not only must the average number of chromosomes contributed by a given ancestor, taken as a type, be calculated, but also the possible range in number of chromosomes contributed must be stated.

But besides those worked out on the accompanying pedigree-charts, there are many other mathematically measurable features of ancestral influence. For instance, the probability of transmitting from a given ancestor to the F_1 zygote not only a definite number but a definite combination of specific chromosomes, also the probability of paternity or maternity of descent of particular chromosomes to the ancestor who passes them on, can be formulated.

Ultimately the biological analysis of a human pedigree will resolve itself into tracing specific chromosomes and parts of chromosomes as the physical determiners of traits, from the propositus back through the family-tree. Thus if, as seems probable, there are but 12 chromosomes in each human gamete, then each half-pedigree, regardless of how many individuals there are in the ancestral generation, can contribute but 12 chromosomes. For example, in the paternal and maternal half-pedigrees, in the P_5 generation there are, in each, sixteen direct ancestors; then (barring crossing-over) at least four of these ancestors must be left out entirely so far as chromosomal contribution, and consequently ancestral influence, is concerned. Of course each ancestor is physiologically necessary as a nurse for the germ-plasm, even though in the course of descent all of the chromosomes of a particular ancestor be shunted in the processes of gametogenesis. The location in the pedigree, of the ancestor supplying the gamete which presents a crossing-over in one of the chromosomes, may be established when the knowledge of linkage and the linear geography of chromosomes becomes more exact. Thus until chromosomes are actually weighted as to their relative ontogenetic influence, we must divide "blood" not into halves, quarters, eighths, sixteenths and so on, by the usual genealogical method, but into twenty-four uneven and individual parts. While it is thus proper for genealogical purposes to speak of an individual as fifteen-sixteenths white and one-sixteenth negro, to be biologically correct reference should be to an individual carrying so many (and which) of the total of twenty-four chromosomes from white ancestors and so many from black. It is perfectly proper to speak of a human individual as seven twenty-fourths or nineteen twenty-fourths of one race, provided the twenty-four units are conceived of as individual and, possibly, very unequal elements. With the weighting of chromosomes and the evaluation of fractional chromosomes in crossing-over, the fractions measuring "blood" or ancestral influence will possess no standard denominators, unless perchance such denominator be the ultimate number of human genes.

In any particular zygote there is of course no chance that a given ancestor will contribute anything except what he has actually contributed. The ratios shown on the accompanying pedigree-charts are measures of probability in reference to events which have not yet happened, that is, measures applicable to descendants not yet born. The averages refer not to specific individuals, but to the occupant of a definite position in the pedigree, taken as a type. The ratio of an accompanying formula may be viewed also in the light of an even or a safe bet or probability,

if we be ignorant of the facts in a specific case, that a given ancestor contributed thus and so from his germ-plasm to the given zygote.

Biologically the formulae here given are as sound as current biological knowledge. They may be modified and developed in accordance with the advance of biological learning. They are equally as applicable to any normally bisexual species of plant or animal as to man.

Mathematically the accompanying formulae are built upon the following elementary principles of (a) probability, and (b) combination.

(*a*) *Probability*

1. The certainty that an event will occur is measured by the quotient 1:1.

2. The impossibility of an event occurring is measured by the quotients 0:∞ or 1:∞.

3. If out of n possible happenings, there are no conditions favoring the happening of one possibility over another, the probability that a given event will occur is $1:n$.

4. If out of n possible happenings, there are no conditions favoring the happening of one possibility over another, the probability that a given event will not occur is $1:\frac{n}{n-1}$.

5. The probability that two or more events will occur is measured by the product of their respective probability-quotients, provided that between or among the several possibilities there is no definite dependence or interference.

6. The probability that none of two or more independent events will occur is measured by the product of the quotients of probability that each event separately will not occur.

7. The probability that a given complex (of certain independent events happening and certain other independent events not happening) will eventuate, is measured by the continued product of the several quotients of probability that each particular constituent event as chosen will or will not occur.

8. The probability that a single possible event will occur within a given portion of a definite range of possibilities is measured by the sum of the probability-quotients of the separate chances constituting the particular given portion.

9. The probability that a single possible event will not occur within a given portion of a definite range of possibilities is $1:\frac{n}{n-h}$, in which

n = the total number of equal chances in the range.
h = the number of equal chances in the given portion.

(*b*) *Combinations*

c = whole number of combinations possible within n factors taken m at a time.

$$c = \frac{n(n-1)\,(n-2)\,\ldots\,(n-m+1)}{1.2.3\ldots.m}.$$

n = whole number of factors.
m = number of factors taken at a time (never greater than n).

FORMULAE FOR CALCULATING ANCESTRAL INFLUENCE IN BISEXUAL SPECIES

The values of formulae number 1, 4, 5, 6, 7, 8, 9 and 10 for each individual to whom the particular formulae apply are given on the accompanying charts showing the ancestral influence in the human male, and in the human female. (Plates 1 and 2.)

1. A = average number of chromosomes passed on by the selected ancestor, taken as a type, to the F_1 zygote, i.e., the propositus.

A = also the average number of chromosome-pairs each of which contributes one member of the diploid or somatic group of chromosomes of the F_1 zygote.

In man the diploid or somatic number of chromosomes is taken tentatively (see explanatory note, page 436) at 24, i.e. 11 equivalent pairs and one XX or XY sex pair.

$A = \frac{n}{2^{(p-1)}}$ + (C_y *or* C_x in case of ancestors from whom an X or a Y chromosome *may* descend.)

See plates 1 and 2 for X- and Y-trails.

2. n = number of equivalent chromosome-pairs (i.e., excluding the XY or XX sex-pair) characteristic of the species. In the case of man n is taken as 11.

3. p = the numerical value of the sub-figure of P used in pedigree-manner to indicate the ancestral or parental generation in relation to the propositus or principal figure of the pedigree. Thus in the great-grandparental generation, P_3, $p = 3$.

4. C_y = the average number of Y-chromosomes contributed to the F_1 zygote by the particular *male* ancestor taken as a type.

$1:C_y$ = the probability that a particular *male* ancestor will contribute the single Y-chromosome found in the F_1 *male* zygote.

The value of $1:C_y$ is always 1:1, and it is applicable to the *direct male* line only. For an ancestor not in the Y-trail, $1:C_y = 1:\infty$.

This direct paternal line down which the *Y*-chromosome descends is the so-called "Y-trail."

5. C_x = the average number of X-chromosomes contributed to the F_1 zygote by the particular ancestor taken as a type.

$1:C_x$ = the probability that the particular male or female ancestor will contribute an X-chromosome to the F_1 zygote.

The value of C_x is determined by the following rule:

(a) Beginning with the X-chromosomes in the F_1 zygote, without exception (i.e., with the chance of 1:1) the *single* X-chromosome in the *male* is derived from his *mother*; and of the *two* X-chromosomes in the *female*, *one* is derived from *each parent*.

(b) In the P_1 and all older generations, in the case of a female zygote the chance of the descent of an X-chromosome from the *father* or *mother* is *equal* (i.e., 1:2), but in the *male* the X-chromosome is always received from the *mother* alone (i.e., the chance is 1:1).

Therefore, the probability that a given ancestor, taken as a type, will contribute an X-chromosome to the F_1 zygote, (i.e., $1:C_x$), is equal to the product of all probability ratios, made according to rules (a) and (b) above, along the X-trail from the selected ancestor to the P_1 generation inclusive.

Thus $1:C_x$ for the mother's mother's father of a female zygote = (1:1) . (1:2) . (1:2) = 1:4; but for the mother's father's mother of a female zygote $1:C_x$ = (1:1) . (1:2) . (1:1) = 1:2.

For an ancestor not in the X-trail, $1 : C_x = 1:\infty$.

Thus, the so-called "X-trail"—from a *son to the mother only*, but from a *daughter to both the father and mother*, traces the several lines in the ancestral pedigree down *one* of which an X-chromosome *must* descend.

NOTE: The X-trail and the Y-trail and all calculations herein based upon them apply only to those species in which the male is the heterozygous and the female the homozygous sex. In species in which the reverse is true the X- and the Y-trails exchange positions. The general rule is that the Y-trail follows the heterozygous sex; the X-trail from the homozygous individual to both parents, but from a heterozygous individual to the homozygous parent only.

6. R = range in number of chromosomes (i.e., in number of single members of chromosome-pairs) possible for the selected ancestor, taken as a type, to contribute to the 24 (i.e., 11 equivalent pairs and 1 XY or 1 XX pair) chromosomes of the F_1 zygote.

In a parent of the P_1 generation, $R = 12 \rightarrow 12$, i.e., there is no range in the number of chromosomes which the given parent may contribute to the F_1 zygote; this number is always 12. For all ancestors of the P_2 and all older generations, $R = 0 \rightarrow 11$, except (a) for those ancestors from whom an X- or a Y-chromosome *must* certainly descend (solid line X-trail or Y-trail on chart), in whom $R = 1 \rightarrow 12$; except also (b) for those ancestors from whom an X-chromosome *may*, by alternative chance, descend (dotted line X-trail in chart), in whom $R = 0 \rightarrow 12$.

In a general formula 11 would be replaced by n, and 12 by $n + 1$, with the value of n as in formula 2.

7. $1{:}C_t$ = the probability (barring mutation, crossing over and other special phenomena) that *one or the other* member of *each* of *all* of the *equivalent* chromosome-pairs characteristic of the species (i.e., an entire chromosome-series excluding the XX and XY pairs), from the given ancestor will enter the F_1 zygote.

In the case of ancestors not in the X-trail or the Y-trail, such an occurrence is the extreme limit of ancestral influence possible to a given ancestor, and its happening precludes any hereditary influence at all on the propositus, i.e., the F_1 zygote, on the part of any other ancestor of the same paternal or maternal half-pedigree, as the case may be, except the lineal ancestors and descendants of the given ancestor; consequently $1{:}C_t$ also measures the probability that the selected ancestor will contribute all of the representatives of the equivalent chromosome-pairs entering the F_1 zygote from the particular half-pedigree and generation.

$1{:}C_t = 1{:}(2^{(p-1)})^n$

n = value as in formula 2. In man n is taken as 11.

p = value as in formula 3.

NOTE: Formula 7 is in fact a special case of formula 17.

8. $1:C_{tx}$ or $1:C_{ty}$ = the probability (barring mutation, crossing over and other special phenomena) that *one or the other* member of *each* of the several equivalent chromosome-pairs characteristic of the species, and *also* one X- or one Y-chromosome, as the case may be, from the given ancestor will enter the F_1 zygote. (In man this total gametic number of chromosomes is taken as 12.)

Such an occurrence can happen only in reference to ancestors in the X- or Y-trails, and its happening precludes any hereditary influence at all on the propositus, i.e., the F_1 zygote, on the part of any other ancestor of the same paternal or maternal half-pedigree, except the lineal ancestors and descendants of the given ancestor.

$1:C_{tx} = (1:C_t) \,.\, (1:C_x)$

Values of $1:C_t$ and $1:C_x$ as in formulas 5 and 7.

$1:C_{ty} = (1:C_t) \,.\, (1:C_y)$

Values of $1:C_t$ and $1:C_y$ as in formulas 4 and 7.

9. $1:C_{-t}$ = the probability (barring mutation, crossing over and other special phenomena) that *no member* of *any* of the equivalent chromosome-pairs characteristic of the species (thus barring for the time being all consideration of the XY or XX chromosome-pairs) from the given ancestor will enter the F_1 zygote.

In such an occurrence the given ancestor and all of his lineal ancestors are *entirely eliminated* as contributors to the natural inheritance of the propositus, i.e., the F_1 zygote, *provided* that such given ancestor is not in the X-trail or Y-trail, and does not, due to inbreeding, appear elsewhere in the ancestral pedigree.

$$1:C_{-t} = 1:\left(\frac{2^{(p-1)}}{2^{(p-1)}-1}\right)^n$$

n = value as in formula 2.

p = value as in formula 3.

$1:C_{-t}$ measures the probability that a selected ancestor will contribute none of the representatives of the equivalent chromosome-pairs entering the F_1 zygote from the particular half-pedigree.

Therefore, the probability that the selected ancestor will contribute all of the representatives of the equivalent chromosome-pairs equals the probability that all of the remaining members of the same ancestral generation of the same half-

pedigree will be left out entirely as contributors of members of equivalent chromosome pairs to the F_1 zygote; whence the check for formula 7,

$$1{:}C_t = 1{:}\,(C_{-t}) \cdot (2^{(p-1)} - 1)^n$$

in which $2^{(p-1)} - 1$ is always the number of ancestors of the particular generation of the same half-pedigree who contribute nothing in case the one remaining member contributes all, and in which n equals the number of equivalent chromosome-pairs characteristic of the species.

Still more generally the probability that a given combination of members of the same ancestral generation of the same half-pedigree will contribute no representative of the equivalent chromosome-pairs to the F_1 zygote equals $1{:}\,(C_{-t}) \cdot m^n$ in which m is the number of members of the selected combination of ancestors of the same generation and the same half-pedigree which cannot exceed $2^{(p-1)} - 1$.

NOTE; It is apparent that the probability that a given ancestor will contribute *no* member of any equivalent chromosome pairs to the F_1 zygote (formula 9), is quite different from the probability that a given ancestor *will not contribute all* of the chromosome representative of the entire chromosomal series. The latter is the negative of formula 7, which negative is stated, $1{:}\,\frac{(2^{(p-1)})^n}{(2^{(p-1)})^n - 1}$

NOTE: Formula 9 is in fact a special case under formula 19.

10. $1{:}C_{-tz}$ or $1{:}C_{-ty}$ = the probability (barring mutation, crossing over and other special phenomena) that *no member* of *any* equivalent chromosome-pair, *nor* of the XX or XY chromosome-pair, from the given ancestor will enter the F_1 zygote, i.e., the probability (barring crossing over and other special phenomena, and the appearance of the given ancestor elsewhere in the pedigree) of the *complete elimination* of the hereditary influence of the given ancestor and all of his or her lineal ancestors, on the propositus, i.e., the F_1 zygote.

This formula is applicable *only* to those ancestors *in the X-trail or Y-trail.*

$1{:}C_{-tz} = (1{:}C_{-t}) \cdot (1{:}C_{-z})$

Values of $1{:}C_{-t}$ and $1{:}C_{-z}$ as in formula 9 above and 11 following.

$1{:}C_{-ty} = (1{:}C_{-t}) \cdot (1{:}C_{-y})$

Values of $1{:}C_{-t}$ and $1{:}C_{-y}$ as in formula 9 above and 11 following.

11. $1{:}C_{-x}$ = the probability that a given ancestor who is in the X-trail will *not* contribute an X-chromosome to the F_1 zygote.

$$1{:}C_{-x} = 1{:}\frac{C_x}{C_x - 1}$$

For an ancestor not in the X-trail, $1{:}C_{-x} = 1{:}1$.
Value of C_x as in formula 5.

12. $1{:}C_{-y}$ = the probability that a given ancestor who is in the Y-trail will *not* contribute a Y-chromosome to the F_1 zygote.

$$1{:}C_{-y} = 1{:}\frac{C_y}{C_y - 1}$$

For an ancestor not in the Y-trail, $1{:}C_{-y} = 1{:}1$.
Value of C_y as in formula 4.

13. $1{:}G_a$ = the probability that *one or the other* member of an allelomorphic pair of Mendelian genes (disregarding genes in the XX or XY sex-chromosomes), regardless of whether the selected member is dominant or recessive to its allelomorph, from the given ancestor will enter the F_1 zygote.

$1{:}G_a = 1{:}2^{(p-1)}$

$G_a = 2^{(p-1)}$ = the number of ancestors in a given half-generation.

$1{:}G_a$ = also the probability that the *entire chromosome*, (barring crossing over, non-disjunction, and other special phenomena), of which the particular gene is a part will enter the F_1 zygote.

p = value as in formula 3.

NOTE: This ratio may well be called the foundation formula of human heredity. As biological knowledge advances other subsidiary formulae may be deduced and synthesized with it.

14. $1{:}G_{-a}$ = the probability that *neither member* of a given allelomorphic pair of Mendelian genes (disregarding genes in the XX or XY sex-chromosomes) from the given ancestor will enter the F_1 zygote.

$$1{:}G_{-a} = 1{:}\frac{1}{1 - \frac{1}{2^{(p-1)}}}$$

p = value as in formula 3.

$1{:}G_{-a}$ = also the probability that *neither* member of the *entire chromosome-pair*, barring crossing over, non-disjunction and other special phenomena, of which the particular gene is a part, will enter the F_1 zygote.

15. $1{:}G_b$ = the probability that a *definitely selected paternally or maternally contributed* member of an allelomorphic pair of Mendelian genes (disregarding genes in the XX or XY sex-chromosomes),

regardless of whether the particular member is dominant or recessive to its allelomorph, from the given ancestor will enter the F_1 zygote.

$1:G_b = 1:2^p$

$1:G_b$ = also the probability that, barring crossing-over, non-disjunction and other special phenomena, the *entire chromosome*, of which the particular gene is a part will enter the F_1 zygote.

p = value as in formula 3.

16. $1:G_{-b}$ = the probability that a *definitely selected paternally or maternally contributed* member of a pair of Mendelian genes, (disregarding genes in the XX or XY sex-chromosomes), from the given ancestor will *not* enter the F_1 zygote.

$$1:G_{-b} = 1:\frac{1}{1-\frac{1}{2^p}}$$

p = value as in formula 3.

$1:G_{-b}$ = also the probability that, barring crossing over, non-disjunction and other special phenomena, the *entire chromosome* of which the particular gene is a part will *not* enter the F_1 zygote.

17. $1:C_a$ = the probability (barring mutation, crossing over and other special phenomena) that *one or the other* member from each of a *definitely selected combination* of equivalent chromosome-pairs (i.e., a definite combination among the chromosomes a to k, if 11 be the number of such equivalent pairs in man, thus disregarding the alternative XX and XY sex-pairs), from the given ancestor, regardless of the behavior of other chromosome-pairs, will enter the F_1 zygote.

$1:C_a = 1:(2^{(p-1)})^a$

p = value as in formula 3.

a = the number of such chromosomes in the definitely selected combination, (which in man cannot exceed 11).

$1:(2^{p-1})^a$ = also the probability that a *definite combination of one of the other allelomorphs* of non-linked genes, barring crossing over, non-disjunction and other special phenomena, carried by the given ancestor, will enter the F_1 zygote.

18. $1:C_b$ = the probability (barring mutation, crossing over, and other special phenomena) that a *definitely selected paternally or maternally* contributed member from *each* of a *definitely selected combination* of equivalent chromosome-pairs (i.e., a definite combination among chromosomes a to k, if 11 be the number

of such equivalent pairs in man, thus disregarding the alternative XX and XY sex-pairs) from the given ancestor, regardless of the behavior of other chromosome-pairs, will enter the F_1 zygote.

$1:C_b = 1:(2^p)^b$

p = value as in formula 3.

b = number of definitely selected paternally or maternally descended chromosomes in the definite combination passed on by the given ancestor.

$1:(2^p)^b$ = also the probability that a definite combination of definitely selected paternally or maternally descended *non-linked* genes, barring crossing over, non-disjunction and other special phenomena, carried by the given ancestor will enter the F_1 zygote.

19. $1:C_{-c}$ = the probability that *neither* member of any pair of a *definitely selected* combination of equivalent chromosome-pairs (i.e., a definite combination among chromosome-pairs a to k, if 11 be the correct number in man, thus disregarding the alternative XX and XY sex-pairs) from the given ancestor, regardless of the behavior of other chromosome-pairs, will enter the F_1 zygote.

$$1:C_{-c} = 1:\frac{1}{\left(1-\frac{1}{2^{(p-1)}}\right)^c}$$

c = number of chromosomes in the definitely selected combination barred from the F_1 zygote. In man it must not be greater than 11.

p = value as in formula 3.

$1:C_{-c}$ = also the probability that *neither* allelomorph of a *definitely selected combination* of *non-linked* Mendelian genes, barring crossing over, non-disjunction and other special phenomena, carried by the given ancestor will enter the F_1 zygote.

20. $1:C_d$ = the probability (barring mutation, crossing-over and other special phenomena) that *one or the other member* of a *definitely selected combination* of equivalent chromosome-pairs (definite combinations among the chromosome-pairs a to k, if 11 be the correct number of equivalent chromosome-pairs in man, thus disregarding the alternative XX and XY sex-pair), and *no more* chromosomes from the given ancestor will enter the F_1 zygote.

$$1{:}C_d = 1{:}\frac{(2^{(p-1)})^a}{\left(1-\frac{1}{2^{(p-1)}}\right)^c}$$

a = value as in formula 17.
n = value as in formula 2.
p = value as in formula 3.
c = value as in formula 19.

NOTE: This formula is the product of formula 17 times formula 19, in which always the number of chromosomes entering plus the number barred from the F_1 zygote = n, i.e., $a + c = n$.

21. $1{:}C_e$ = the probability (barring mutation, crossing over and other special phenomena) that a *definitely selected paternally or maternally* contributed member from each of a *definitely selected combination* of equivalent chromosome-pairs (i.e., a definite combination among chromosome-pairs a to k, if 11 be the number of such equivalent pairs in man, thus disregarding the alternative XX and XY sex-pairs), and *no more* chromosomes, from the given ancestor, will enter the F_1 zygote.

$$1{:}C_e = 1{:}\frac{(2^p)^b}{\left(1-\frac{1}{2^{(p-1)}}\right)^c}$$

b = value as in formula 18.
n = value as in formula 2.
p = value as in formula 3.
c = value as in formula 19.

NOTE: This formula is the product of formula 18 times formula 19, in which always the number of chromosomes entering plus the number barred from the F_1 zygote = n, i.e., $a + c = n$.

22. $1{:}N_a$ = the probability (barring mutation, crossing over, and other special phenomena) that a *definite number* (but no more) of chromosomes from the given ancestor, regardless of their individuality, and of their paternal or maternal descent, will enter the F_1 zygote.

$$1{:}N_a = 1{:}\frac{\dfrac{(2^{(p-1)})^d}{\left(1-\frac{1}{2^{(p-1)}}\right)^c}}{\dfrac{n\cdot(n-1)\cdot(n-2)\cdot(n-3)\ldots.(n-d+1)}{1\cdot 2\cdot 3\ldots. d}}$$

d = the *definite number* of chromosomes (regardless of combinations) passed on to the F_1 zygote.

n = value as in formula 2.

p = value as in formula 3.

$c = n - d$.

23. $1 : N_b$ = the probability (barring mutation, crossing over and other special phenomena) that a *definite number* (but no more) of chromosomes from the given ancestor, regardless of their individuality, but *specified in total numbers as to the maternity and paternity* of their descent, will enter the F_1 zygote.

$$1 : N_b = 1 : \frac{\dfrac{(2^{(p-1)})^d \cdot 2^d}{\left(1 - \dfrac{1}{(2^{(p-1)})}\right)^c}}{\dfrac{n \cdot (n-1) \cdot (n-2) \cdot \ldots . (n-d+1)}{1 \cdot 2 \cdot 3 \ldots . d} \times \dfrac{d \cdot (d-1) \cdot (d-2) \ldots . (d-s+1)}{1 \cdot 2 \cdot 3 \ldots . s}}$$

d = value as in formula 22.

n = value as in formula 2.

p = value as in formula 3.

$c = n - d$.

s = the number of chromosomes originating in the selected parent of the given ancestor, that will enter the F_1 zygote.

$d - s$ = the number of chromosomes originating in the other parent of the given ancestor, that will enter the F_1 zygote.

24. In case of inbreeding or consanguineous matings, the measure of ancestral influence (barring mutation, crossing over, and other special phenomena) is determined as follows:

(a) A_m = the average number of chromosomes which a given ancestor, taken as a type, who appears more than once in the direct ancestry, contributes to the propositus.

A_m = the sum of the several values of A for each of the several appearances of the particular ancestor in the pedigree.

(b) R_m = the range in possible number of chromosomes which a given ancestor, taken as a type, who appears more than once in the direct ancestry, contributes to the propositus.

$R_m = r' \rightarrow r''$ (i.e., from r' to r'').

r' = the *highest* of the several *minima* in the several values of R for the given ancestor in his or her several places in the direct ancestry.

r'' = the *highest* of the several *maxima* in the several values of R for the given ancestor in his or her several places in the direct ancestry.

(c) $1{:}C_{imz}$ = the probability that a given ancestor, taken as a type, who appears more than once in the direct ancestry, will contribute from *one or more* of the several such appearances *all* of the chromosomes supplied by the particular half-pedigree to the propositus.

$$1{:}C_{imz} = 1{:}\ \frac{\left(\frac{h}{g}\right)^n}{(1{:}C_x)\cdot(1{:}C_y)}$$

n = value as in formula 2.

h = the total number of direct ancestors in the oldest half-generation in which the particular inbred ancestor appears ($=2^{(p-1)}$).

p = value as in formula 3.

g = the number of parts of the particular half-pedigree covered by the given ancestor, in terms of h units.

C_x = value as in formula 4.

C_y = value as in formula 5.

(d) $1{:}C_{-imz}$ = the probability that a given ancestor, taken as a type, who appears more than once in the direct ancestry will contribute *no* chromosomes to the propositus.

$$1{:}C_{-imz} = 1{:}\ \left(\frac{\frac{h}{g}}{\frac{h}{g}-1}\right)^n \cdot (C_{-xa}\cdot C_{-xb}\cdots)\cdot(C_{-y})$$

n = value as in formula 2.

h = value as in formula 24(c).

g = value as in formula 24(c).

C_{-x} = value as in formula 11. (a, b, and so on, following the x in the formula, refers to C_{-x} in the first, second and other appearances of the propositus in the X-trail).

C_{-y} = value as in formula 12.

It is obvious that 24(c) or 24(d), if in the given case the *last* two parentheses be omitted, measures the given probability in reference to all of the chromosomes except the X and Y sex-members. Also if n represent a definite combination of n

specific chromosomes, regardless of the maternity or paternity of their descent and regardless of the behavior of other chromosomes, the formula without the last two parentheses is true for the specific chromosomal combination selected. Also these same formulae without the *first* parenthesis measure the given probability for the X and Y sex-chromosomes.

(e) The probability that the propositus will be a zygotic chromosomal replica of a given ancestor who appears once or more in both the paternal and maternal half-pedigrees is equal to the product of the probability-quotient that the particular inbred ancestor will supply all of the chromosomes contributed to the F_1 zygote by the paternal half-pedigree (formula 24(c)) *times* the same probability-quotient in reference to the maternal half-pedigree *times* $1:2^{(n+1)}$.

n = value as in formula 2.

It is obvious that in the case of species with XY-type of male and XX-type of female a male replica can occur only if the repeating ancestor is a male and appears at least in both the X- and Y-trails; the female replica only if the repeating ancestor is a female and appears at least in the X-trail in both the paternal and maternal sides of the house. The formula, however, takes all of these chances into consideration.

A zygotic chromosomal replica in relation to its repeated ancestor is biologically the equivalent of one identical twin to its mate, although in the case of chromosomal replicas the natural traits have been segregated, distributed among several direct ancestors and later reassembled in the replica, while in the case of identical twins the duplication is immediate from the zygote.

25. S = the minimum number of ancestors in a given ancestral generation among whom will be found all of the possible contributors of the sex-chromosomes (i.e., XX or XY) to the F_1 zygote.

p = the number of the given ancestral generation: e.g., for parents, $p = 1$; for grandparents, $p = 2$; for great-grandparents, $p = 3$; and so on as in formula 3.

In the human female in which the sex-chromosome formula is X + X, S = the $(p + 3)$th member of the Fibonacci series. The maternal half-pedigree provides one X-chromosome in which half-pedigree S = Fibonacci serial term $p + 2$. In the paternal half-pedigree which also contributes one X-chromosome, S = Fibonacci serial term $p + 1$.

In the human male the maternal half-pedigree contributes an X-chromosome, the paternal half-pedigree a Y-chromosome. In this sex for both half-pedigrees united, S = (Fibonacci serial term $p + 2$) + 1. In the maternal half pedigree S = Fibonacci serial term $p + 2$, in the paternal half-pedigree S = 1.

Fibonacci series = 0, 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, ..., in which each term is the sum of the two preceding terms.

SUMMARY

1. The present problem of measuring ancestral influence by tracing chromosomes or gene-radicals is demonstrated to be a special case under the following general situation:

Given:

(a) A single biological unit factor (a gene) in a known situation (in a P_1 ancestor) and the quotient measuring the probability of its reacting in a definite manner or reaching a definite new situation at the end of a single move or transformation (in the F_1 zygote).

(b) The demonstrated synchronization or sequence of action of two or more such biological unit factors (several genes through several generations).

Solution:

By applying the correct principles of combination and chance, the probability that a given complex situation will result from a given set of constituent conditions at the end of a given number of definite kinds of transformations is mathematically formulated.

In the case of measuring ancestral influence by tracing chromosomal descent, the situations and transformations concern definite chromosome-complexes in the zygote and their breaking-up, their recombination and their transmission to offspring in accordance with the principles demonstrated by genetics and cytology.

2. The formulae of bisexual heredity here set forth are foundational. They are stated in general terms whose validity depends upon their presenting correct mathematical pictures of the chromosomal processes which work out in gametogenesis and fertilization.

3. When knowledge of a special case or type of chromosomal behavior is worked out, the mathematical formula which describes the particular case may be joined to the foundational formulae here given, and thus the the general mathematical formula of bisexual heredity may be gradually approximated.

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LEGEND FOR ♂ CHROMOSOME-ORIGIN CHART (PLATE 1)

NOTES: The calculations on this chart (a) are based upon the theory that the zygotic chromosome-formula for the human male is (11 + 1X) + (11 + 1Y), and (b) do not account for mutation, crossing over, special schemes of selection or other special phenomena.

The formulae upon which these calculations are based are general and foundational, and apply to any bisexual species for which the chromosome-number is given. They may be corrected to account for special phenomena by the articulation of formulae for particular processes when the latter are mathematically generalized.

A = average number of chromosomes passed on by the selected ancestor to the F_1 zygote. (Formula 1.)

ΣA for all of the ancestors of a given generation = 24.

R = range within the haploid number of chromosomes (i.e., number of single members of chromosome pairs) possible for the selected ancestor to contribute to the 24 (i.e., 11 equivalent pairs and the XY sex-pair) chromosomes of the F_1 zygote. Barring crossing over each actual contribution is a whole number. (Formula 6.)

C_1 = the chance that one or the other member of each equivalent chromosome pair, i.e., an entire haploid series, excluding a member of the XY sex-pair, in a given ancestor will enter the F_1 zygote. (Formula 7.)

C_{-1} = the chance that no member of any equivalent chromosome pair in a given ancestor will enter the F_1 zygote. (Formula 9.)

C_x = the chance that the selected ancestor carried the single X-chromosome that will enter the F_1 ♂ zygote. (Formula 5.)

Also = the average number of X-chromosomes contributed by the selected ancestor to the F_1 zygote.

C_y = the chance that the selected ancestor carried the single Y-chromosome that will enter the F_1 ♂ zygote. (Formula 4.)

Also = the average number of Y-chromosomes contributed by the selected ancestor to the F_1 zygote.

C_{1x} or C_{1y} = the chance that one or the other member of each equivalent chromosome pair and also one X- or Y-chromosome in the selected ancestor, will enter the F_1 zygote, i.e., that the selected ancestor represents all of the ancestral influence of her or his generation in her or his half-pedigree. (Formula 8.)

C_{-1x} or C_{-1y} = the chance that no member of any equivalent chromosome pair nor of the XX or XY sex-pair in the selected ancestor will enter the F_1 zygote, i.e., the chance of complete elimination of the ancestral influence of the selected ancestor. (Formula 10.)

For the F_1 male zygote

Certain selected formula-values for given ancestors in the P_4 generation

(P_4 1)

$A = 1.375 + 1Y$

$R = 1 \rightarrow 12$

$C_{1y} = 1:8,589,934,592$

$C_{-1y} = 0:\infty$

$C_y = 1:1$

(P_4 2, 3, 4, 5, 6, 7, 8, 9, 10, and 13)
$A = 1.375$
$R = 0 \rightarrow 11$
$C_t = 1{:}8{,}589{,}934{,}592$
$C_{-t} = 1{:}4.3442+$
(P_4 11, 12, and 14)
$A = 1.375 + 0.25X$
$R = 0 \rightarrow 12$
$C_{tx} = 1{:}34{,}359{,}738{,}368$
$C_{-tx} = 1{:}5.7922+$
$C_x = 1{:}4$
(P_4 15 and 16)
$A = 1.375 + 0.125X$
$R = 0 \rightarrow 12$
$C_{tx} = 1{:}68{,}719{,}476{,}736$
$C_{-tx} = 1{:}4.9648+$
$C_x = 1{:}8$

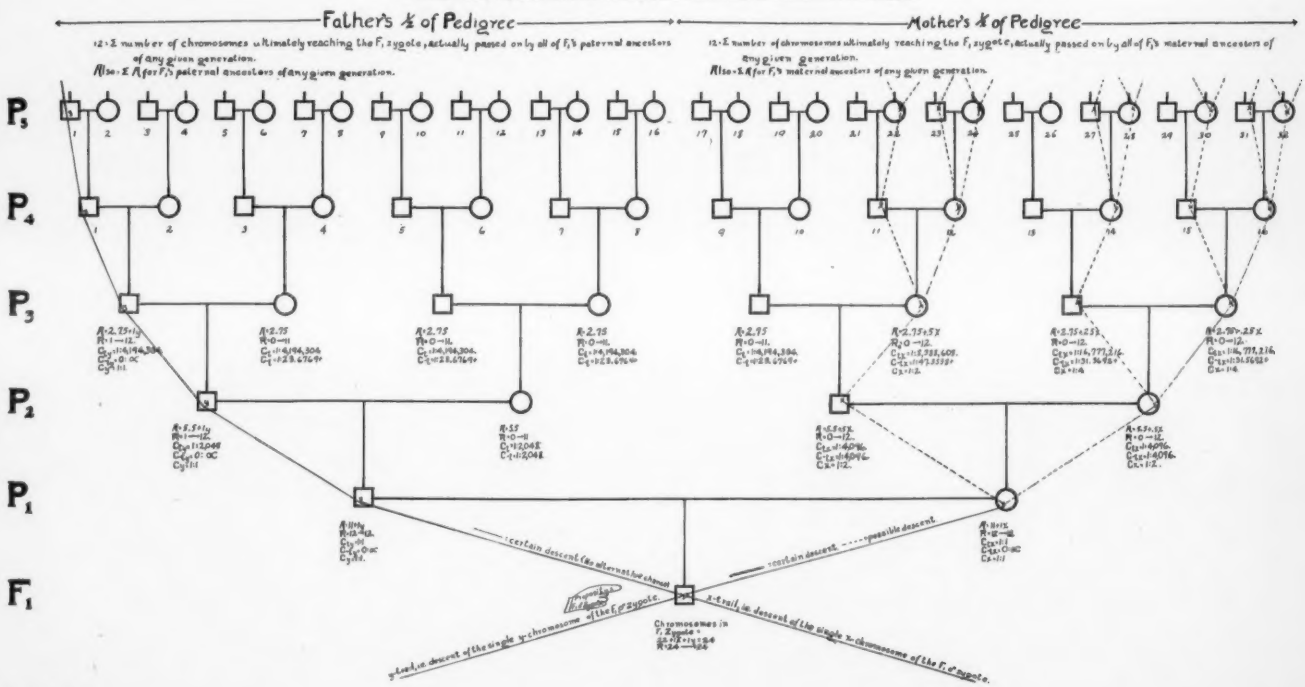
For the F_1 male zygote

Certain selected formula-values for given ancestors in the P_5 generation

(P_5 1)
$A = 0.6875 + 1Y$
$R = 1 \rightarrow 12$
$C_{ty} = 1{:}17{,}592{,}186{,}044{,}416$
$C_{-ty} = 0{:}\infty$
$C_y = 1{:}1$
(P_5 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 25, 26, and 29)
$A = 0.6875$
$R = 0 \rightarrow 11$
$C_t = 1{:}17{,}592{,}186{,}044{,}416$
$C_{-t} = 1{:}2.0338+$
(P_5 22)
$A = 0.6875 + 0.25X$
$R = 0 \rightarrow 12$
$C_{tx} = 1{:}70{,}368{,}744{,}177{,}664$
$C_{-tx} = 1{:}2.7117+$
$C_x = 1{:}4$
(P_5 23, 24, 27, 28, and 30)
$A = 0.6875 + 0.125X$
$R = 0 \rightarrow 12$
$C_{tx} = 1{:}140{,}737{,}488{,}355{,}328$
$C_{-tx} = 1{:}2.3243+$
$C_x = 1{:}8$
(P_5 31 and 32)
$A = 0.6875 + 0.0625X$
$R = 0 \rightarrow 12$
$C_{tx} = 1{:}281{,}474{,}976{,}710{,}656$
$C_{-tx} = 1{:}2.1693$
$C_x = 1{:}16$

ANCESTRAL INFLUENCE IN THE HUMAN MALE

Based upon (a) average, (b) range, and (c) chance, in the contribution of chromosomes to the F_1 zygote


Father's ½ of Pedigree
Mother's ½ of Pedigree
Σ number of chromosomes ultimately reaching the F1 zygote, actually passed on by all of F1's paternal ancestors of any given generation.
Also Σ R for F1's paternal ancestors of any given generation.
Σ number of chromosomes ultimately reaching the F1 zygote, actually passed on by all of F1's maternal ancestors of any given generation.
Also Σ R for F1's maternal ancestors of any given generation.
P5
P4
P3
P2
P1
F1
certain descent (the alternative chromosome)
certain descent
possible descent
Chromosomes in F1 zygote:
y-trait in descent of the single y-chromosome of the F1 zygote.
x-trait in descent of the single x-chromosome of the F1 zygote.

LEGEND FOR ♀ CHROMOSOME-ORIGIN CHART (PLATE 2)

NOTE: These calculations (a) are based upon the theory that the zygotic chromosome-formula for the human female is (11 + 1X) + (11 + 1X), and (b) do not account for mutation, crossing over, special schemes of selection or other special phenomena.

A = average number of chromosomes passed on by the selected ancestor to the F_1 zygote. (Formula 1.)

ΣA for all of the ancestors of a given generation = 24.

R = range within the haploid number of chromosomes (i.e., the number of single members of chromosome pairs) possible for the selected ancestor to contribute to the 24 (i.e., 11 equivalent pairs and an XX pair) chromosomes of the F_1 zygote. Barring crossing over each actual contribution is a whole number. (Formula 6.)

C_t = the chance that one or the other member of each equivalent chromosome-pair (i.e., an entire haploid series excluding the X-chromosome) in a given ancestor will enter the F_1 zygote. (Formula 7.)

$C\text{-}t$ = the chance that no member of any equivalent chromosome pair, excluding the XY sex-pair, in a given ancestor will enter the F_1 zygote. (Formula 9.)

C_x = the chance that the selected ancestor carried one of the X-chromosomes that will enter the F_1 ♀ zygote. (Formula 5.)

Also = the average number of X-chromosomes contributed by the selected ancestor to the F_1 zygote.

Ctx = the chance that one or the other member of each equivalent chromosome-pair and also one X-chromosome in the selected ancestor will enter the F_1 zygote, i.e., that the selected ancestor represents all of the ancestral influence in her or his generation in her or his half-pedigree. (Formula 8.)

$C\text{-}tx$ = the chance that no member of any equivalent chromosome-pair nor of the XX or XY sex-pair in the selected ancestor will enter the F_1 zygote, i.e., the chance of complete elimination of the ancestral influence of the selected ancestor. (Formula 10.)

For the F_1 female zygote

Certain selected formula-values for given ancestors in the P_4 generation

(P_4 1, 2, 3, 4, 5, 9, 10, and 13)

$A = 1.375$
$R = 0 \rightarrow 11$
$C_t = 1{:}8{,}589{,}934{,}592$
$C\text{-}t = 1{:}4.3442+$

(P_4 6)

$A = 1.375 + 0.5X$
$R = 0 \rightarrow 12$
$Ctx = 1{:}17{,}179{,}869{,}184$
$C\text{-}tx = 1{:}8.6884+$
$C_x = 1{:}2$

(P_4 7, 8, 11, 12, and 14)

$A = 1.375 + 0.25X$
$R = 0 \rightarrow 12$
$Ctx = 1{:}34{,}359{,}738{,}368$
$C\text{-}tx = 1{:}5.7922+$
$C_x = 1{:}4$

(P_4 15 and 16)

$A = 1.375 + 0.125X$
$R = 0 \rightarrow 12$
$Ctx = 1{:}68{,}719{,}476{,}736$
$C\text{-}tx = 1{:}4.9648+$
$C_x = 1{:}8$

For the F_1 female zygote

Certain selected formula-values for given ancestors in the P_8 generation

(P_8 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 17, 18, 19, 20, 21, 25, 26, and 29)

A = 0.6875
R = 0 → 11
C_t = 1:17.592,186,044,416
C_{-t} = 1:2.0338+

(P_8 11, 12, 14, and 22)

A = 0.6875 + 0.25X
R = 0 → 12
Ctz = 1:70,368,744,177,664
C_{-tz} = 1:2.7117+
Cz = 1:4

(P_8 15, 16, 23, 24, 27, 28, and 30)

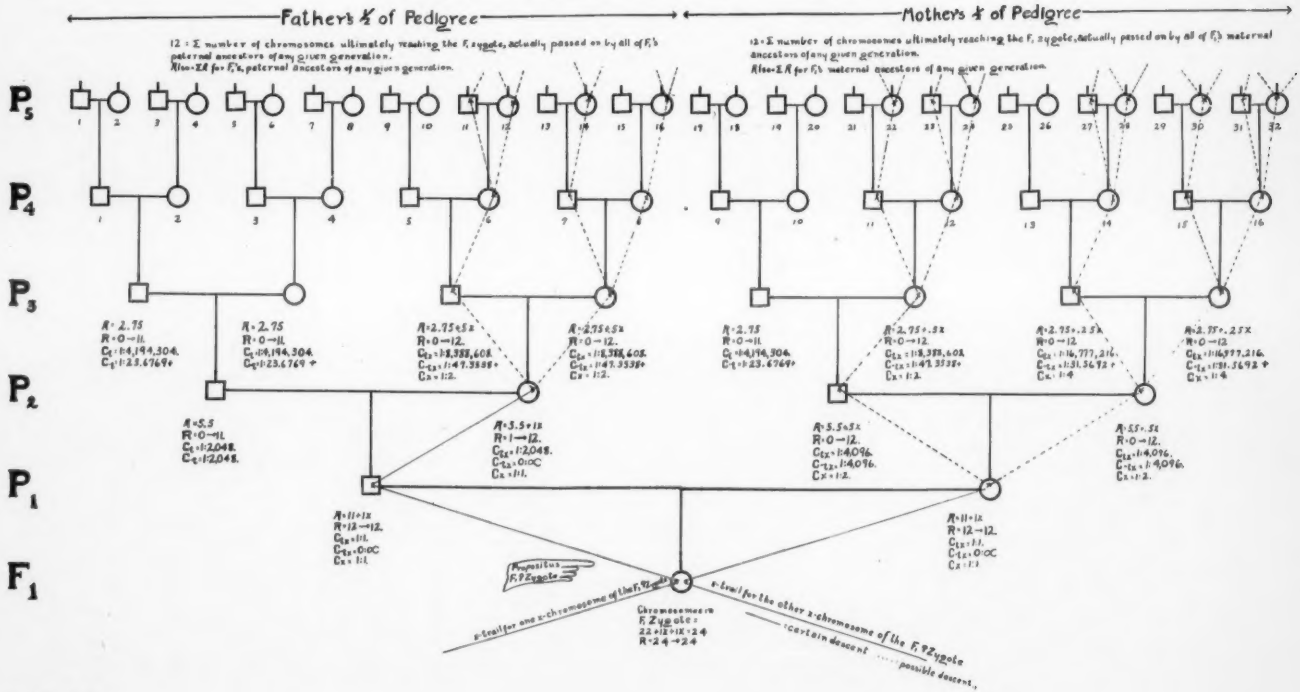
A = 0.6875 + 0.125X
R = 0 → 12
Ctz = 1:140,737,488,355,328
C_{-tz} = 1:2.3243+
Cz = 1:8

(P_8 31 and 32)

A = 0.6875 + 0.0625X
R = 0 → 12
Ctz = 1:281,474,976,710,656
C_{-tz} = 1:2.1693+
Cz = 1:16

ANCESTRAL INFLUENCE IN THE HUMAN FEMALE

Based upon (a) average, (b) range, and (c) chance, in the contribution of chromosomes to the F_1 zygote


Father's ½ of Pedigree
Mother's ½ of Pedigree
12 = Σ number of chromosomes ultimately reaching the F1 zygote, actually passed on by all of F1's paternal ancestors of any given generation.
Also = Σ R for F1's paternal ancestors of any given generation.
12 = Σ number of chromosomes ultimately reaching the F1 zygote, actually passed on by all of F1's maternal ancestors of any given generation.
Also = Σ R for F1's maternal ancestors of any given generation.
P5
P4
P3
P2
P1
F1
A = 2.75 R = 0→11 Cx = 1:4,194,304. Cx = 1:23.6769+
A = 2.75 R = 0→11 Cx = 1:4,194,304. Cx = 1:23.6769+
A = 2.75±2.5 R = 0→12. Cx = 1:2,388,608. Cx = 1:47.3538+ Cx = 1:2.
A = 2.75±2.5 R = 0→12. Cx = 1:2,388,608. Cx = 1:47.3538+ Cx = 1:2.
A = 2.75 R = 0→11 Cx = 1:4,194,304. Cx = 1:23.6769+
A = 2.75±2.5 R = 0→12. Cx = 1:2,388,608. Cx = 1:47.3538+ Cx = 1:2.
A = 2.75±2.5 R = 0→12. Cx = 1:16,777,216. Cx = 1:31.3692+ Cx = 1:4
A = 2.75±2.5 R = 0→12. Cx = 1:16,777,216. Cx = 1:31.3692+ Cx = 1:4
A = 5.5 R = 0→11. Cx = 1:2,048. Cx = 1:2,048.
A = 3.5±1.5 R = 1→12. Cx = 1:2,048. Cx = 1:0.0C Cx = 1:1.
A = 5.5±3.5 R = 0→12. Cx = 1:4,096. Cx = 1:4,096. Cx = 1:2.
A = 5.5±3.5 R = 0→12. Cx = 1:4,096. Cx = 1:4,096. Cx = 1:2.
A = 11±1.2 R = 12→12. Cx = 1:1. Cx = 1:0.0C Cx = 1:1.
A = 11±1.2 R = 12→12. Cx = 1:1. Cx = 1:0.0C Cx = 1:1.
Propositus F.1 Zygote
x-trait for one x-chromosome of the F1 zygote
x-trait for the other x-chromosome of the F1 zygote
Chromosomes in F1 Zygote: 22+12+1X+1Y = 24 R = 24→24
certain descent
possible descent.

GENETIC AND CYTOLOGICAL EXAMINATION OF THE PHENOMENA OF PRIMARY NON-DISJUNCTION IN *DROSOPHILA MELANOGASTER*

SHELLEY R. SAFIR

Columbia University, New York City

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INTRODUCTION

The phenomena of non-disjunction, including the production of "exceptions" to sex-linked inheritance, take their origin in the failure of members of the pair of sex-chromosomes to segregate into separate gametes (BRIDGES 1913, 1914, and especially 1916). In individuals of the normal chromosomal constitution, such as XX females, non-disjunction was found to be of very rare occurrence; only about one egg in 1700 was found to be of the XX type or conversely of the no-X type. If such an XX egg produced by primary non-disjunction in a normal female is fertilized by a normal Y sperm then a matroclinous daughter of the constitution XXY is produced. The existence of XXY females has been demonstrated by BRIDGES both by direct cytological examination and by conclusive genetic tests. Matroclinous XXY females produce further exceptions, which are called secondary, to the extent of about 4.3 percent.

The cause of this production is the fact that the presence of the extra Y chromosome with its characteristic synaptic behavior forces both X's to enter the same egg in a certain percent of reductions. In his latest article on non-disjunction (1916) BRIDGES has reported very thorough genetic and cytological tests of the various phenomena of secondary non-disjunction, and his results furnish convincing proof of the chromosome theory of heredity.

In addition to the production of XXY females, primary non-disjunction was found to have other interesting results. The fertilization of the no-X egg by an X sperm was found by BRIDGES to give rise to a patroclinous son of the chromosome type XO. Such XO males proved to be entirely normal in somatic appearance both as to sex-linked characters (though "exceptions" to sex-linked inheritance) and as to sexual characters, but such XO males produced by primary non-disjunction were found to be absolutely sterile.

It was foreseen that adequate study of primary non-disjunction would be very difficult because of the fact that the cause or causes of primary non-disjunction were purely conjectural, and one must therefore depend upon the chance occurrence of primary non-disjunction for the working material.

At the suggestion of Prof. T. H. MORGAN and Dr. C. B. BRIDGES I undertook the following problems in connection with primary non-disjunction:

1. Large-scale tests of the frequency of occurrence of primary non-disjunction.

2. A study of the frequencies of primary non-disjunction in diverse stocks.

3. More precise tests of the degree and generality of the sterility of the patroclinous XO males produced by primary non-disjunction.

4. The cause or causes of such sterility.

5. Cytological demonstration of the constitution of the sterile patroclinous males produced by primary non-disjunction.

THE FREQUENCY OF PRIMARY NON-DISJUNCTION

In an experiment to test the constitution of patroclinous sons in secondary non-disjunction BRIDGES (1916) found 12 primary exceptions in a total of 20,484 flies. Various other experiments gave further information on the frequency of primary non-disjunction which seemed to be in the neighborhood of one in 2000 offspring.

For my more extensive tests of the frequency of primary exceptions, begun in the summer of 1914, flies homozygous for the two sex-linked recessive mutants, eosin eye color and miniature wings, were out-crossed

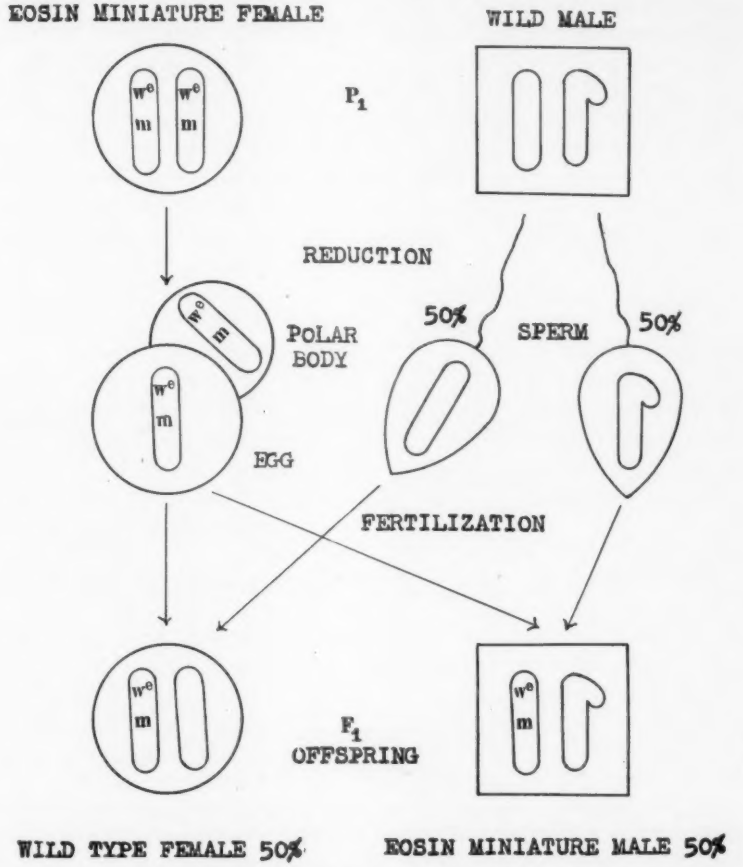


FIGURE 1.—Diagram illustrating normal criss-cross inheritance characteristic of sex-linkage. The genes for the two sex-linked mutations, eosin and miniature, are carried by the X chromosome (straight), the Y chromosome (J-shaped) is neutral with respect to these mutant characters.

to wild males. Normally when this cross is made all the sons, deriving their single X chromosome from their mother, show the characters,—in this case eosin and miniature,—whose genes are carried by the X

chromosome of the mother. The daughters, however, fail to show these recessive sex-linked characters because the normal dominant allelomorphs of eosin and minature are brought in by the X chromosome from the father. Figure 1 shows a diagram of the normal course of inheritance in the above cross.

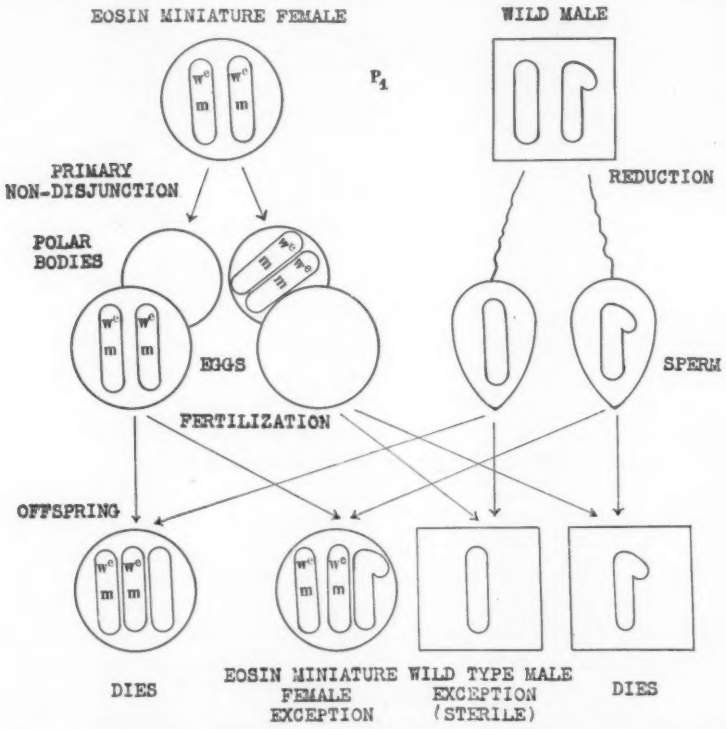


FIGURE 2.—Diagram to show primary non-disjunction of the X chromosomes in an eosin miniature female and the exceptions produced when the resulting XX and zero eggs are fertilized by X and Y sperm of a wild male.

In addition to these regular eosin miniature males and wild-type females two other classes of offspring, eosin miniature females and wild-type males, appeared in very small numbers. The appearance of these exceptions is due to the occurrence of primary non-disjunction. In a very small percent of the eggs, during the reduction division, the two X chromosomes fail to disjoin quickly enough and are both caught either in the egg

or in the polar body, so that two abnormal types of eggs are produced. One type has two X chromosomes and correspondingly the other type has no X. Such eggs when fertilized by the X and Y sperm of a wild male give four abnormal types of zygotes XXX, XO, XXY, and YO. BRIDGES showed that both the YO and XXX zygotes are not viable, and hence fail to give any actual class among the progeny. Of the two remaining classes, the XXY becomes an exceptional matroclinous eosin-eyed, miniature-winged female, since both of her eosin miniature X chromosomes have come from the mother. The XO individual is a male since it has only one X, and is patroclinous, since this single X comes from his wild-type father.

These exceptions, produced by an XX female, are known as primary exceptions and the process which is responsible for their production is named primary non-disjunction. Figure 2 gives a diagram of the method of production of such exceptional gametes and zygotes.

Since in the experiments of BRIDGES the frequency of the occurrence of exceptions due to primary non-disjunction had been found to be in the neighborhood of one-twentieth of one percent, it was apparent that very large numbers would be required in order to secure data at all adequate. At first a long series of matings were started in which 245 cultures were raised from matings of single virgin eosin miniature females with one (or two) wild males.

TESTS OF THE NATURE OF THE EXCEPTIONS OF TABLE 1

An examination of the different cultures of table 1 makes it apparent that the results are in general very uniform. The great majority of the 245 cultures, namely 168, contained no exceptions at all, which is in accordance with a low frequency of production of primary exceptions. About a quarter of the cultures (namely 67) contained one exception each. In these 67 cultures the percentage of exceptions is so low that in all, except a few of the smallest, this fact alone is sufficient to prove that the exceptions in question were descended from XX mothers and were accordingly primary. Only seven cultures produced two exceptions each, and here again the percentage of exceptions (about one percent) is so low as to make it improbable that they are the result of secondary non-disjunction, which averages 4.3 percent of exceptions. Two cultures produced three exceptions each and one culture produced five exceptions. The culture which produced five exceptions (No. 261) is distinguished also by the fact that three of these exceptions were females. Both the

TABLE 1

Offspring given by eosin miniature females when out-crossed to wild males. Exceptions that were themselves tested by breeding are marked with an asterisk (*). Those cultures which were tested by tests of about 8 regular daughters are marked with a dagger (†).

No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions	
	♂ +	♀ w	♂ w	♀ +		♂ +	♀ w	♂ w	♀ +		♂ +	♀ w	♂ w	♀ +
1	64	56	—	—	53	62	41	—	—	*†106	102	95	—	1
2	82	79	—	—	56	45	62	—	—	107	101	97	—	—
3	60	67	—	—	57	68	60	—	—	108	103	97	—	—
*5	86	90	—	1	60	94	60	—	—	†109	76	70	—	1
6	91	83	—	—	*62	59	55	1	—	110	124	128	—	—
7	61	42	—	—	63	52	48	—	—	111	119	129	1	—
*†8	65	47	—	1	†65	75	56	—	1	112	101	89	—	—
*†9	84	83	1	—	*†67	85	77	—	1	113	121	138	—	—
10	103	108	—	—	69	84	73	—	1	114	103	96	—	—
12	82	66	—	—	70	108	97	—	—	115	98	107	—	—
*13	126	100	—	1	71	141	101	—	—	116	131	101	1	—
*†14	48	34	—	1	73	134	123	—	—	†117	144	101	—	1
17	93	79	—	—	74	103	121	—	1	118	105	98	—	—
18	45	48	—	—	75	84	75	—	—	120	110	85	—	—
*†19	72	85	—	2	76	88	76	—	—	121	119	92	—	1
21	48	27	—	—	77	98	101	—	—	122	131	111	—	—
23	76	75	—	—	78	118	130	—	—	123	128	116	—	—
*25	72	44	1	—	79	59	65	—	—	125	95	96	—	—
*†26	115	89	—	1	†81	95	87	—	1	†126	102	92	—	1
*27	84	69	1	—	83	115	112	—	—	128	138	129	—	—
29	27	22	—	—	84	102	100	1	—	129	163	147	—	—
*30	130	101	—	1	85	128	109	—	1	130	93	99	—	—
31	120	115	—	—	86	88	91	—	—	133	101	107	—	—
32	136	104	—	—	87	111	100	—	—	134	126	119	—	—
34	120	104	—	1	88	102	97	—	—	135	113	72	—	—
35	46	38	—	—	89	145	138	—	—	136	111	121	—	—
36	51	48	—	—	90	107	91	—	—	*†137	80	63	—	1
37	79	69	—	—	91	100	103	—	—	140	112	108	—	—
38	67	69	—	—	92	117	109	—	—	142	104	121	—	—
39	89	87	—	—	†93	112	101	—	1	143	118	70	—	—
40	51	52	—	—	95	119	132	—	1	*†144	100	89	—	1
†41	51	43	—	1	96	112	122	—	—	145	105	97	—	—
43	48	47	—	—	97	125	110	—	—	146	108	69	—	—
44	48	46	—	—	98	177	165	—	—	148	119	114	—	—
45	121	117	—	1	99	113	116	—	—	151	101	101	—	1
47	69	63	—	—	100	120	108	—	—	152	105	97	—	—
48	82	62	—	—	*102	114	126	—	2	153	105	92	—	—
*49	75	75	1	—	103	103	91	—	—	*154	85	98	—	2
50	138	117	—	—	104	99	90	—	—	*†155	142	109	—	1
51	156	129	—	—	105	107	131	—	—	156	95	85	—	—

TABLE 1 (continued)

No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions	
	♂ +	♀ w a	♂ w a	♀ +		♂ +	♀ w a	♂ w a	♀ +		♂ +	♀ w a	♂ w a	♀ +
158	99	103	1	—	213	109	108	—	—	263	79	97	—	—
159	108	91	—	1	214	135	135	—	—	*264	77	62	1	—
160	121	102	—	—	*215	118	125	1	—	265	100	85	—	1
161	113	94	—	—	216	83	69	—	1	266	107	83	—	—
*163	110	85	—	1	218	81	75	—	—	267	113	87	—	—
164	117	90	—	—	219	128	113	—	—	268	107	112	—	—
165	120	134	—	—	220	103	97	—	—	269	61	60	—	—
168	101	77	—	—	221	75	85	—	1	*271	79	73	—	2
169	98	98	—	—	222	167	113	—	—	272	99	101	—	—
170	104	71	—	1	223	80	84	—	—	273	104	98	—	—
173	167	98	—	—	225	90	89	—	1	274	75	72	—	—
174	102	91	—	—	226	106	91	—	—	275	70	74	—	—
*175	111	86	—	2	227	132	115	—	—	*276	105	86	—	1
176	94	88	—	—	*†228	162	129	1	2	277	100	82	—	—
177	124	85	—	1	229	121	111	—	—	279	98	71	—	—
*†178	79	67	—	1	230	82	52	—	—	280	107	103	—	—
179	86	79	—	—	231	99	96	—	—	*281	104	101	—	2
180	114	98	—	1	232	117	105	—	—	282	64	66	—	—
181	98	110	—	—	234	96	128	—	—	283	64	67	—	1
182	103	112	—	1	235	140	114	—	—	*284	118	94	1	1
183	87	67	—	—	237	132	120	—	—	285	108	101	—	—
186	114	58	—	1	238	129	120	—	1	286	106	105	—	—
187	132	90	—	—	239	105	102	—	—	287	101	95	—	—
188	122	86	—	—	240	139	126	—	—	288	93	94	—	—
189	129	85	—	—	241	123	113	—	1	289	106	101	1	—
192	133	98	—	—	243	90	68	—	—	290	104	75	—	—
193	103	86	—	—	244	92	111	—	—	293	123	110	—	—
*†194	106	99	—	3	*†245	118	85	1	—	294	121	102	—	—
195	114	110	—	—	*†246	91	84	—	1	295	108	100	1	—
196	86	70	—	1	247	113	116	—	1	296	101	108	—	—
198	95	92	—	—	248	92	74	—	—	298	79	62	—	1
199	106	97	—	—	249	118	121	—	—	299	101	94	—	—
200	139	108	—	—	250	109	62	—	—	300	61	52	—	1
201	116	121	—	—	251	105	82	—	—	301	101	98	—	—
202	102	107	—	—	252	90	101	—	—	303	57	39	—	—
203	106	76	—	—	*253	151	125	—	1	*304	101	94	—	1
205	131	118	—	1	254	97	77	—	—	305	100	86	—	—
206	95	93	1	—	256	100	95	—	1	306	77	60	—	—
207	149	137	—	—	257	123	86	—	—	307	102	107	—	—
208	152	138	—	—	259	102	102	—	—	310	42	22	—	—
209	92	76	—	1	260	112	103	—	—					
210	122	104	—	—	*†261	126	98	3	2	Total*	25,004	22,454	17	70
212	145	126	—	—	262	115	93	—	—					

*Exclusive of 261.

large number of exceptions and the equality of sexes mark this culture as the product of secondary non-disjunction. Further specific tests proved this to be true (see tables 2 and 9). In the case of the two cultures (194 and 228) that produced three exceptions each, the percentages of exceptions were still so low (1.5 and 1.0 percent, respectively), that they were probably due to primary non-disjunction, judging solely from that fact. However, in these cases certain other tests proved the mothers to have been XX in constitution and the exceptions primary (see tables 1 and 3).

The difference between the percentage of exceptions characteristic of primary and of secondary non-disjunction (0.05 percent versus 4.3 percent, according to the experiments of BRIDGES) was recognized as insufficient for a conclusive test of the constitution of the eosin miniature females. This source of confusion was foreseen and certain other tests were applied. BRIDGES had found that every male which from the pedigrees should have been a primary exception was absolutely sterile while the XY exceptional males produced by secondary non-disjunction

TABLE 2

Offspring given by the two exceptional sons from culture 261 when tested by wild females

No.	Wild-type ♀	Wild-type ♂
261a	112	101
261b	127	119

behaved precisely like normal males and were correspondingly fertile. The number of primary exceptions that BRIDGES tested (15) while not sufficient to prove that all XO males are sterile, yet offered a test that could be applied tentatively to males suspected of being XO in constitution. The cultures in table 1 in which the sterility test was applied to exceptional males are marked with an asterisk. It was expected that the two males of culture 261 would prove fertile as in fact they did (table 2), proving in a third manner that that culture was an example of secondary non-disjunction. In all the other tests, however, the exceptional males proved absolutely sterile. The validity of this sterility test has since been completely justified (see page 000) so that we may regard the 34 cultures in which the exceptional males were sterile as being of proved XX descent.

The third test applied is the most conclusive of all since it depends upon the fact, established by BRIDGES, that half the regular daughters of an XXY female are themselves XXY and produce further secondary

exceptions of the usual frequency (4.3±). Since the presence of the extra Y makes no somatic difference in the fly, enough such daughters must be tested to be sure of including at least one such XXY daughter. According to the laws of probability, where two classes occur in equal frequency, selection at random of one individual gives an even chance of securing the desired type, selection of two individuals gives a 3:1 chance of success and so on up. With 8 selections the chance of failure to include an XXY daughter would be one in 256 or practically negligible.

There were two other considerations that made tests by this method very desirable. As soon as a culture has been proved to be descended from an XX mother, that fact establishes the XX nature of each test culture descended from her, and accordingly each such set of test cultures furnishes about eight additional cultures in which all exceptions are known to be primary without the necessity of further tests. These test cultures, then, furnish highly valuable information on the frequency of primary non-disjunction. The other, and relatively minor consideration, was that these regular daughters were heterozygous for both eosin and miniature and their regular sons would furnish large amounts of data on the crossing over between these two loci. In order that there might be no confusion between the regular and the exceptional offspring in these tests, either in the female or in the male offspring, the tests were made by out-crossing to males carrying the dominant sex-linked mutation, bar. All of the exceptional daughters would then be wild type, the exceptional sons bar, the regular daughters bar (of the heterozygous type), and the regular sons would all be not-bar, but would be in four classes with respect to eosin and minature, as required by the known amount of crossing over between these loci.

Table 3 gives the results of the tests of several daughters from each of 23 of the cultures of table 1 that had given exceptions. While eight such cultures were started in each case, as expected, accidents or poor food or other unfavorable conditions cut down the final number in many cases.

In all, 162 daughters were tested which represented 23 original cultures of table 1. Of these sets of tests one only gave evidences of secondary non-disjunction, and this simply added a fourth proof of the XXY nature of the mother of culture 261. Of the twelve daughters of 261 seven gave secondary exceptions and five gave no exceptions. These five are accordingly included in the grand total of XX cultures of table 3. The remaining sets of cultures in each case proved that the mothers

TABLE 3

Offspring given by wild-type daughters from table 1 when out-crossed to bar males

	B	Regular sons				Exceptions	
		$w^e m$	+	w^e	m	+♀	B♂
8	136	41	55	26	18	—	—
	86	40	27	15	14	—	—
	162	49	47	16	21	—	—
	60	21	15	12	9	—	—
	103	35	32	15	19	—	—
	125	39	24	21	21	—	—
Total	672	225	200	105	102	—	—
9	96	29	30	10	16	—	—
	86	32	29	17	15	—	—
	103	33	38	17	19	—	—
	129	40	35	16	17	—	—
	155	46	45	27	21	—	—
	168	49	50	22	29	—	1
Total	737	229	227	109	117	—	1
14	109	41	50	14	17	1	—
	109	32	27	8	11	—	—
	90	30	29	17	12	—	—
	144	50	43	23	24	—	—
	112	26	33	15	18	—	—
	72	22	16	11	10	—	—
Total	636	201	198	88	92	1	—
19	118	37	46	23	15	—	—
	124	37	44	15	11	—	—
	111	32	39	21	16	—	—
	86	25	38	10	10	—	—
	126	45	46	15	18	—	1
	35	14	14	3	5	—	—
	34	11	11	8	6	—	—
	100	26	28	16	19	—	—
Total	734	227	266	111	100	—	1
26	206	63	68	23	24	—	—
	154	44	45	26	20	—	—
	125	32	50	18	15	—	1
	103	38	47	18	19	—	—
Total	588	177	210	85	78	—	1
41	95	24	40	10	17	—	—
	151	47	41	21	21	—	—
	88	30	40	16	18	—	—
	119	42	34	19	27	—	—
	167	46	50	20	29	—	—
	79	27	22	9	11	—	—
41	155	58	50	19	21	—	—
	149	33	43	23	22	—	—
Total	1003	307	320	137	166	—	—
65	108	32	35	21	18	—	—
	103	38	28	15	16	—	—
	129	40	33	16	17	—	—
	106	44	38	23	19	—	—
	103	36	32	15	14	—	—
	75	32	24	8	14	—	—
Total	624	222	190	98	98	—	—
67	99	31	30	15	12	—	—
	70	31	33	12	19	—	—
	82	28	22	11	10	—	—
	101	33	39	16	18	—	—
	116	42	41	19	22	—	—
Total	468	165	165	73	81	—	—
81	217	67	77	31	32	—	—
	252	95	76	37	35	—	—
	221	81	92	33	36	—	—
	218	68	92	35	31	—	—
	166	50	56	26	25	—	—
	187	66	59	24	39	—	1
	194	70	62	29	36	—	—
	191	68	73	20	30	—	—
Total	1646	565	587	235	264	—	1
93	143	47	60	24	26	—	—
	98	50	34	18	16	—	—
	170	49	52	21	33	—	—
	223	81	72	27	36	—	—
	221	71	76	22	24	—	1
	190	70	67	26	29	—	—
	233	71	76	31	31	—	—
	205	74	68	20	27	—	—
Total	1483	513	505	189	222	—	1
106	203	59	84	37	33	—	—
	182	45	65	26	19	—	—
	239	70	74	25	25	—	—
	194	42	63	31	31	—	1
	158	52	61	24	28	—	1
	158	50	55	18	22	—	—
	171	40	49	30	26	—	—
	235	85	80	23	27	—	—
Total	1540	443	531	214	211	—	2

TABLE 3 (continued)

	B	Regular sons				Exceptions	
		w m	+	w^e	*m*	+♀	*B*♂
109	293	106	109	54	49	—	—
	155	57	55	18	18	—	—
	216	77	82	32	35	—	—
	168	57	51	20	21	—	—
	128	43	46	17	22	—	—
	212	90	65	31	36	—	—
	156	70	55	16	29	—	—
Total	1328	500	463	188	210	—	—
117′	192	63	59	19	34	—	—
	157	80	61	21	24	—	—
	227	85	100	35	36	—	—
	174	56	62	27	31	—	—
	252	96	89	37	37	—	—
	193	71	60	39	32	—	—
	129	47	40	19	23	—	—
	183	72	63	26	22	—	—
Total	1507	570	534	223	239	—	—
126	194	76	69	28	29	—	—
	159	56	65	20	32	—	—
	149	45	43	18	22	—	—
	65	24	17	11	7	—	—
	201	75	59	32	38	—	—
	186	60	45	18	19	—	—
	162	70	52	15	18	—	—
	252	92	87	41	41	—	—
Total	1368	498	437	183	206	—	—
137	206	64	54	37	24	—	—
	173	50	55	21	16	—	—
	177	54	78	24	27	—	—
	244	83	76	37	28	—	—
Total	800	251	263	119	95	—	—
144	219	66	59	17	27	—	—
	176	54	56	19	20	—	—
	196	61	61	31	34	—	—
	158	64	64	20	30	—	—
	150	50	53	21	18	—	—
	187	62	52	25	21	—	—
	181	81	68	25	24	—	1
Total	1267	438	413	158	174	—	1
155	227	97	73	47	40	—	—
	230	74	85	39	41	—	1
	226	82	71	38	44	—	—
	191	81	78	25	31	—	—
	69	31	26	10	12	—	—
155	200	74	61	23	38	—	—
	219	65	75	31	35	1	—
	178	61	55	26	16	—	—
Total	1540	565	524	239	257	1	1
178	233	81	76	34	35	—	—
	132	37	41	19	24	—	—
	134	38	45	16	18	—	—
	165	51	54	23	22	—	—
	157	64	57	27	24	—	—
	112	43	46	22	19	—	1
	163	45	65	31	20	—	—
Total	1096	359	384	172	162	—	—
194	203	66	71	28	25	—	1
	156	44	45	25	20	—	—
	153	50	53	22	22	—	—
	163	55	56	23	27	—	—
	136	44	51	19	25	—	—
	114	43	38	16	18	—	—
	194	67	76	35	32	—	—
	189	58	68	23	25	—	—
Total	1308	427	458	191	194	—	1
228	203	68	84	26	31	—	1
	168	47	56	28	20	—	—
	239	83	88	29	42	—	—
	222	81	67	22	21	1	—
	176	73	58	13	24	—	—
	186	60	74	26	23	—	—
Total	194	412	427	144	161	1	1
245	135	57	46	23	21	—	—
	173	64	65	24	23	—	—
	155	55	49	23	24	—	1
	181	52	52	19	17	—	—
	121	40	38	26	21	—	—
	159	60	68	23	24	—	—
Total	924	328	313	138	130	—	1
246	197	55	71	26	28	—	—
	85	25	33	17	20	—	—
	226	73	70	36	33	—	—
	193	72	63	29	31	—	1
	197	80	66	27	27	—	—
	224	84	72	33	35	—	—
	196	77	56	24	29	1	—
	160	53	50	19	17	—	—
Total	1478	519	481	211	220	1	1

TABLE 3 (concluded)

	B	Regular sons				Exceptions			B	Regular sons				Exceptions	
		$w^e m$	+	w^e	m	+♀	B♂			$w^e m$	+	w^e	m	+♀	B♂
261	111	35	31	21	17	3	4	261	116	39	42	21	19	—	—
	86	26	28	15	13	1	3		91	29	28	16	13	—	—
(XXY)	73	30	26	14	16	2	1	(XX)	118	42	36	22	29	—	—
	112	37	40	18	22	8	6		83	26	34	13	17	—	—
	121	45	46	21	18	6	9		80	28	32	14	17	—	—
	104	33	40	18	19	3	3	Total	488	164	172	86	95	—	—
	132	46	51	22	23	5	6	Grand							
Total	739	252	262	129	128	28	32	total*	24,429	8305	8268	3496	3674	4	14

* Exclusive of 261 (XXY).

of these cultures and of the parent culture (marked with a † in table 1) are all of the constitution XX and the exceptions are all primary.

With the completion of these tests we are in position to calculate the percentages of exceptions due to primary non-disjunction in table 1. There were 87 exceptions in a grand total of 47,545, or one in 546 flies; that is, 0.18 percent. Likewise the grand totals for the XX cultures of table 3 show that there were 14 patroclinous male exceptions and 4 exceptional daughters in a total of 48,190 flies or one in 2,677 flies; that is, 0.04 percent. The frequency of primary exceptions in these cultures was found to be about that in the experiments of BRIDGES and apparently lower than in my eosin miniature experiments, though whether the difference is large enough to be significant will be discussed in connection with further experiments on that point.

A very curious and significant relation came to light when the number of female and of male exceptions were compared. The number of such males ran far ahead of the corresponding females in both tables 1 and 3 and the total number of males, 84, was four times as great as the total number of females, 21. This feature will be discussed in a later section.

FREQUENCY OF PRIMARY EXCEPTIONS PRODUCED BY VERMILION FEMALES AND BY WHITE FEMALES

To determine whether the frequency of primary non-disjunction was different in different stocks, as might seem to be suggested by the difference between the results of BRIDGES'S experiments (1 in about 2,000), the eosin miniature test (1 in about 550), and the test of regular daughters

(1 in about 2,600), a second and a third series of tests were carried out on the unrelated stocks of the sex-linked recessive mutants vermilion eye color and white eye color. Table 4 gives the results of the tests of 93 vermilion females from stock by out-crossing to wild males.

TABLE 4

Offspring given by vermilion females when out-crossed to wild males

Culture number	Regular offspring		Exceptions		Culture number	Regular offspring		Exceptions		Culture number	Regular offspring		Exceptions	
	+♀	v♂	v♀	+♂		+♀	v♂	v♀	+♂		+♀	v♂	v♀	+♂
1001	154	94	—	—	1039	146	135	—	—	1076	153	130	—	—
1002	110	106	—	—	1041	157	159	—	—	1077	80	77	—	—
1003	103	89	—	—	1042	167	131	—	—	*1078	143	157	—	1
1004	127	128	—	—	†*1043	116	121	—	2	1079	121	106	—	—
1006	159	174	—	—	†1044	101	61	1	—	1080	156	134	—	—
1007	114	98	—	—	1045	157	164	—	—	1081	132	126	—	—
†*1009	155	149	—	1	1047	115	114	—	—	1082	121	113	—	—
1010	159	138	—	—	1048	193	180	—	—	1084	145	82	—	—
†*1011	121	70	—	1	1049	174	115	—	—	1085	134	83	—	—
*1012	144	122	—	1	1050	199	160	—	—	*1086	119	115	—	1
1013	140	134	—	—	1051	39	24	—	—	1087	143	103	—	—
1014	123	110	—	—	1052	173	142	—	—	1088	106	91	—	—
1015	93	68	—	—	1053	157	139	—	—	1089	129	82	—	—
1016	157	135	—	—	1054	162	121	—	—	1090	103	108	—	—
†*1017	102	108	—	1	1055	157	144	—	—	1091	126	46	—	—
1018	180	113	—	—	*1056	144	156	—	1	†*1092	138	117	—	1
†*1019	136	104	—	1	1057	150	129	—	—	1093	82	58	—	—
1022	89	62	—	—	1058	195	162	—	1	*1094	141	130	—	1
1023	210	179	—	—	1059	152	181	—	—	1095	108	108	—	—
†1024	138	132	1	—	1060	210	157	—	—	1096	145	170	—	—
1025	110	86	—	—	1061	39	33	—	—	1097	106	101	—	—
1026	147	100	—	—	1062	145	153	—	—	1098	108	104	—	—
†*1027	79	54	—	1	*1064	117	92	—	1	1099	76	75	—	—
1028	164	103	—	—	1065	152	136	—	—	1100	138	130	—	—
*1029	144	160	4	6	1066	161	109	—	—	1101	141	101	—	—
1031	117	105	—	—	1067	171	154	—	1	1102	140	116	—	—
1032	200	177	—	—	1069	136	156	—	—	1103	126	104	—	—
*1034	92	93	—	1	1070	166	132	—	—	1104	163	102	—	—
1035	102	92	—	—	1071	138	125	—	—	1105	123	113	—	—
1036	135	115	—	—	1072	142	138	—	—					
1037	118	95	—	—	1074	120	111	—	—	Total‡	12,348	10,621	2	17
1038	108	108	—	—	1075	65	64	—	—					

‡ Exclusive of culture 1029.

The same sort of tests were carried out with these cultures as with those of table 1 in order to determine if any of the exceptions were due to secondary non-disjunction. It is immediately apparent that culture

1029 with ten exceptions (3.3 percent) is due to secondary non-disjunction. The sterility test was applied to the six exceptional males of culture 1029 and all proved fertile (table 5) as they should if of the supposed XY type.

TABLE 5

Offspring given by the six exceptional sons from culture 1029v when tested by wild females

No. 1029	+♀	+♂
1	122	119
2	97	100
3	134	129
4	108	99
5	152	142
6	88	92
Total.....	701	681

TABLE 6

The two kinds of results given by the regular daughters from culture 1029v when tested by bar males

No. 1029v	Regular daughters	Regular sons		Exceptions		Percent of exceptions
	Bar	v♂	+♂	v♀	B♂	
1	110	48	56	2	3	2.3
2	136	71	63	5	4	3.2
3	78	39	36	3	2	3.1
4	150	73	80	6	3	2.9
5	102	59	57	3	5	3.5
6	131	63	78	4	7	3.9
Total.....	707	353	370	23	24	3.1
7	108	65	48	—	—	—
9	129	63	76	—	—	—
10	79	51	43	—	—	—
11	127	68	72	—	—	—
12	58	27	29	—	—	—
Total.....	501	274	268	—	—	—

The sterility test was applied to fifteen exceptional males from the other cultures of table 4, and in every case these males proved sterile (marked with an asterisk).

Tests of the constitution of the mothers of table 4 by means of tests for XXY daughters, were also carried out. In the case of culture 1029 six test cultures gave secondary exceptions (3.1 percent) while five gave no such exceptions (table 6).

Similar tests carried out with the regular daughters of nine other cultures of table 4 (marked with a †) gave in each case proof of the XX constitution of the mothers. In table 7 the sum of each set of such test cultures is given.

The vermilion XX females of table 4 yielded a total of 22,988 offspring of which 19 were exceptions, 17 males and 2 females. This represents one primary exception in 1209 offspring or 0.08 percent.

The tests of the daughters of culture 1029 (table 6) and of the other cultures (table 7) furnished 70 XX cultures with a total of 19,042 flies of which 12 were exceptions, 2 females and 10 males. This represents one primary exception in 1587 offspring or 0.06 percent.

TABLE 7

Summary of tests by out-crosses to bar males, of regular daughters from cultures of table 4 that gave exceptions

Test of culture	No. of ♀♀ tested	Regular daughters	Regular sons		Exceptions	
		B	+	*v*	*v*♀	*B*♂
1029	8	1148	529	536	—	2
1011	7	894	488	448	—	1
1017	8	1121	545	573	1	1
1019	8	1241	638	605	1	1
1024	6	817	404	379	—	1
1027	6	767	368	345	—	2
1043	7	1013	481	483	—	—
1044	8	1135	542	573	—	1
1092	7	986	461	467	—	1
Total.....	65	9122	4456	4409	2	10

PERCENTAGE OF PRIMARY EXCEPTIONS GIVEN BY THE FEMALES OF THE WHITE STOCK

In a third experiment to test the percentage of primary non-disjunction, over a hundred white-eyed females were out-crossed to wild males (table 8).

The white-eyed females yielded 15 exceptions, 13 males and 2 females, in a total count of 21,773 offspring, which represents one exception in 1452 offspring or 0.07 percent.

The secondary matroclinous exceptions of cultures 261 and 1029 were out-crossed to wild males and gave the expected secondary exceptions in all cases (tables 9 and 10).

A summary and a comparison of the data from the various sources upon the frequency of the occurrence of primary exceptions is given in table 11.

TABLE 8

Offspring given by white females when out-crossed by wild males

No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions	
	+♀	w♂	w♀	+♂		+♀	w♂	w♀	+♂		+♀	w♂	w♀	+♂
2001	94	100	—	—	2038	107	109	—	—	2074	140	113	—	—
2002	80	94	—	—	2039	131	132	—	—	2075	128	104	—	—
2003	77	107	—	—	2040	90	94	—	1	2076	118	106	—	—
2004	105	105	—	—	2041	96	94	—	—	2077	117	103	—	—
2006	125	117	—	—	2042	130	130	—	1	2078	131	107	—	—
2007	111	103	—	—	2043	75	57	—	—	2079	116	117	—	—
2008	107	123	—	—	2044	72	73	—	—	2080	115	106	—	—
2009	106	101	—	—	2045	100	104	—	—	2081	109	104	—	—
2010	100	101	—	—	2046	130	87	—	—	2082	146	143	—	—
2011	79	81	—	—	2047	101	89	—	—	2083	116	142	—	—
*2012	90	86	—	1	2048	156	130	—	—	2084	143	94	—	—
2013	76	63	—	—	2049	92	91	—	—	2085	132	161	—	1
2014	96	101	—	—	2051	115	106	—	—	2086	149	127	—	—
2015	89	72	—	—	2052	120	110	—	—	2087	111	93	—	—
2016	84	61	—	—	*2053	131	120	1	1	2088	115	121	—	—
2018	114	98	—	—	2054	73	87	—	—	2089	107	114	—	—
2019	95	90	—	—	2055	141	96	—	1	2090	100	103	—	—
2020	145	144	—	—	2056	130	127	—	—	2091	156	156	—	—
2021	108	52	—	—	2057	165	148	—	—	2092	151	112	—	1
2022	111	55	—	—	2058	124	122	—	—	2093	111	111	—	—
2024	109	99	—	—	2059	123	127	—	—	2094	140	128	—	—
2025	106	102	—	—	2060	130	112	—	—	2095	168	149	—	—
2026	89	88	—	—	*2061	155	125	1	1	2096	140	122	—	—
2027	57	55	—	—	2062	117	92	—	—	*2097	107	115	—	2
*2028	142	119	—	1	2063	152	139	—	—	2098	129	126	—	—
2029	104	87	—	—	2064	112	93	—	—	2099	155	128	—	—
2030	125	112	—	—	2065	115	96	—	—	2100	134	121	—	1
2031	93	95	—	—	2066	111	108	—	—	2101	102	110	—	—
2032	74	111	—	—	2067	103	81	—	—	2102	122	118	—	—
2033	97	89	—	—	2068	123	113	—	—	2103	103	104	—	—
2034	88	79	—	—	2069	126	147	—	—	2104	119	91	—	—
2035	125	100	—	1	2070	80	66	—	—	2105	141	96	—	—
2036	91	77	—	—	2071	70	82	—	—					
2037	69	76	—	—	2073	71	75	—	—	Total	11,308	10,450	2	13

RELATIVE NUMBER OF FEMALE AND MALE EXCEPTIONS

One of the most puzzling facts disclosed by the foregoing experiments is the uniformly lower frequency of the female exceptions. Out of a

TABLE 9

Offspring given by the three exceptional daughters from culture 261 when tested out by wild-type males

No.	Regular offspring		Exceptions		Percent of exceptions
	+♀	$w^e m$♂	$w^e m$♀	+♂	
261a	100	94	2	3	2.6
261b	125	98	1	1	0.9
261c	120	85	3	3	2.9
Total.....	345	277	6	7	2.09

TABLE 10

The offspring given by four vermilion exceptional females when out-crossed by wild males

No.	Regular offspring		Exceptions		Percent of exceptions
	+♀	v♂	v♀	+♂	
	123	140	6	9	5.4
	94	101	5	4	4.4
	139	143	6	8	4.7
	108	118	6	5	4.6
Total.....	464	502	23	26	4.8

TABLE 11

Summary of data upon the frequency of primary non-disjunction

Series	Total offspring	Total females	Total males	Total exceptions	Total ♀ exceptions	Total ♂ exceptions	Ratio of total exceptions	Ratio of ♀ exceptions	Ratio of ♂ exceptions	Percent of exceptions	Percent of ♀ exceptions	Percent of ♂ exceptions
BRIDGES....	20,484	10,635	9,849	12	5	7	1:1707	1:4097	1:2926	0.06	0.02	0.03
$w^e m$.....	47,545	25,021	22,524	87	17	70	1:546	1:2800	1:679	0.18	0.04	0.15
v.....	22,988	12,350	10,638	19	2	17	1:1209	1:11494	1:1352	0.08	0.009	0.07
w.....	21,773	11,310	10,463	15	2	13	1:1452	1:10886	1:1675	0.07	0.009	0.06
$w^e m$ test cases.....	48,190	24,433	23,757	18	4	14	1:2677	1:12047	1:3442	0.04	0.008	0.03
v test cases..	19,042	9,625	9,417	12	2	10	1:1587	1:9521	1:1904	0.06	0.01	0.05

total of 151 exceptions yielded by the eosin miniature, vermilion, and white, females, and the test cases, only 27 were females whereas 124 were males (table 11).

This difference in the numbers of male and female exceptions cannot be explained by simple primary non-disjunction which results from a delayed reduction for the X chromosomes; for when the polar body is formed the entangled X's pass together to one or to the other pole giving two classes of eggs, XX and O, in equal numbers. These eggs when fertilized by the X and Y sperm, present in equal numbers in a normal male, would result in equal numbers of XO male and XXY female exceptions.

Neither will secondary non-disjunction help out, for BRIDGES found that the percentage of secondary exceptions is the same in the females as in the males, which is in sharp contrast to the condition here found.

This excess of males might be accounted for by assuming that the two X's are extruded into the polar body more frequently than retained in the egg nucleus. There seems no *a priori* reason why this should be so, and DONCASTER (1914) reports that his cytological investigations of a somewhat similar sex disturbance in Abraxas show apparently that the direction taken by the sex chromosomes on the polar spindle is at random.

Another explanation that appears to have more adequate support, is that the greater frequency of O eggs is due to some type of "elimination" of the X chromosome that would normally remain in the egg at the maturation division. MORGAN and BRIDGES (1919) report the occurrence of numerous gynandromorphs in Drosophila, the explanation of which they have proved to be an "elimination" of an X chromosome, occurring at an early cleavage division when two X's divide. They show that in this way an XX zygote may give rise to a nucleus with only one X, at the first, second, or some later cleavage. The parts supplied by the descendants from this one-X nucleus are accordingly male, and may constitute a half, a quarter, or some othe frraction of the individual, depending upon the cleavage division at which the X is eliminated. The parts descended from the original XX type of nuclei are female. If lagging were to occur at the reduction division causing the "elimination" of a whole X chromosome, no-X or zero eggs would be produced whenever the mate of the lost X went out into the polar body. The fertilization of such no-X or zero eggs would produce XO zygotes which would account for the excess of exceptional males.

The formation of O eggs by such a loss of the X chromosome that would normally remain in the eggs gives the same kind of end result as does primary non-disjunction. We may suppose that the initial step in this process is some difficulty in the separation of the synapsed X chromosomes. Either they had become mechanically intertwined to such a

degree or in such a fashion that disjunction is more than ordinarily difficult; or the chemical or other forces that had held them in junction were slow to relax in these particular cases. Besides local environmental conditions that would favor such a delay, it seems probable that in the case of the eosin miniature stock there is present a specific chromosome alteration that makes such a condition more frequent.

There is still a third way of explaining the formation of more O than XX eggs. Extreme lagging of the X chromosomes on the middle of the polar spindle, from whatever cause, would mean that the daughter nuclei would be formed without the inclusion of an X in either. Both X's would be eliminated. All such eggs would be of the O type, and when fertilized would lead only to patroclinous, XO exceptional sons. These primary male exceptions would constitute an excess in proportion to the frequency of the occurrence of this kind of elimination.

There is supporting evidence for the occurrence of "elimination" in forms other than Drosophila. Thus in bees the occurrence of gynandromorphs in which the male parts are sometimes maternal and sometimes paternal are fully explained by elimination, but are not both explainable on any of the former theories of gynandromorphism. Some of the gynandromorphs in some of the moths and butterflies can be explained as due to elimination. In certain nematodes (e.g., *Rhabditis nigrovenosum*) the production of the sexual males depends upon the elimination of an X from cells which were of the hermaphrodite or female type. These considerations made it probable that the excess of primary male exceptions is due to the elimination of both X's from the egg. The cases of female exceptions and a corresponding number of male exceptions are due to typical primary non-disjunction followed by the inclusion of both X's in the egg or in the polar nucleus.

This conception of the case brings into line the surprising fewness of females of the XXY type among females picked at random from culture bottles. Only two cultures among the thirty-two tested gave secondary exceptions, which is in conformity with the very rare formation of XX eggs by primary non-disjunctions.

STERILITY OF THE XO MALE PRODUCED BY PRIMARY NON-DISJUNCTION

As already stated, BRIDGES had tested fifteen primary exceptions and had found them all to be sterile. In the tests of the nature of the exceptions in the cultures of tables 1, 4, etc., I tested a total of fifty-four other such males. In many of these cases there was other and independent

evidence of the primary nature of these exceptions. It was hoped that offspring might be obtained from these XO males in order that a race lacking the Y chromosome might be secured and made the basis of further investigations. Accordingly, exceptional efforts were made to favor fertilization. The XO males were entirely normal in appearance, and, as was carefully tested, were normal in their sexual behavior. Courtship and copulation were normal in method and in the time relations (described by STURTEVANT 1915). Old XO males copulated more readily, and copulation was hastened by the odor from previous copulations, as is the normal condition. The females that had been copulated with by XO males laid eggs, which however in no case developed, despite unusual care. Food conditions were kept favorable by the introduction of larvae which prevented the undue growth of bacteria, yeast, and moulds. Larvae from white-eyed flies, or else, fertilized white females from stock were used so that any true offspring of the XO males could be detected by the different eye color. All these efforts failed, and it became apparent that the sterility of the XO males was established.

CAUSE OF STERILITY OF XO MALES

The first problem attacked in an effort to find the cause of such sterility was whether or not the XO male injects sperm during copulation. An examination of the spermathecae dissected from females after such copulation showed that no sperm was present, while copulations by normal XY males introduced an abundance of motile sperm.

Preparations of the genitalia of XO males were made after treatment with KOH which removed the non-chitinous materials. No difference could be detected between these preparations and others of normal fertile males.

Upon dissection of XO males testes were found to be present and to be of normal shape and color, though probably of smaller size. When these testes were teased open, bundles of sperm appeared as in the normal testes; but these bundles remained compact instead of separating out as do normal bundles, and when the bundles were artificially separated it was found that the sperm of the XO male are non-motile. It was found also that considerably less sperm was produced by the XO male than normal. Many such dissections and comparisons were carried out, and it became apparent that the immediate cause of the sterility was the non-motility of the relatively scanty sperm.

CYTOLOGICAL EVIDENCE OF THE OCCURRENCE OF XO MALES

The genetic evidence of BRIDGES and myself leaves no escape from the conclusion that the patroclinous exceptional sons resulting from primary non-disjunction were of the constitution XO instead of XY. It was anticipated that a cytological demonstration of that point would be my most difficult task.

The cytology of Drosophila males has always presented many serious difficulties; Miss STEVENS, for example, made preparations of some 2,000 males with only partial success in the description of the group. Several other workers have made preparations. The best thus far secured are those of BRIDGES, by which he was able to demonstrate the J shape and the larger size of the Y chromosome. In the case of the XO males I was under the further serious handicap of strictly limited material. To secure one male required the examination of 1,000 flies on the average.

BRIDGES had made preparations of five of his XO males, but these showed the same condition as my early preparations, namely, a few bundles of sperm and very little spermatogonial or spermatocyte tissue. These had been made from males that had been tested for their sterility to be sure of their XO character. But males so old as this have nearly finished the spermatogonial and spermatocyte divisions, and are unsuitable, as we found, for cytological examination.

The next step was to omit sterility tests and to make preparations of the freshly hatched exceptional males, relying upon the tests of sisters to prove their XO nature. This procedure yielded little better results than before; for although the fixation (strong Flemming) and the staining (iron haematoxylin) appeared satisfactory, divisions were not found. These preparations showed slightly greater amounts of spermatogonial tissue than the old males had, but from the fact that the most successful preparations of normal males made by BRIDGES were of larvae, in which adult sperm were already present, it was apparent that the earliest stage at which I could distinguish exceptions must be used. There was unfortunately no sex-linked larval character on hand that could be used, so that characters which showed in the pupa were chosen. Matings were made between eosin miniature bar females (eosin miniature females had given the highest percentage or primary exceptions) and wild males. The character bar eyes was used because it was the one that could be earliest and most surely detected in the pupa. Any male pupa with a full round not-bar eye would be the desired exception. Five such males were ultimately secured. In fixing them an important change in technique

was used, namely, to dissect out the testes free from all fat-bodies which interfere with the action of osmic fixation.

In four out of the five, pupal figures were found that were analysable. In two or three there were a number of mitotic figures that were good and there were a few that can be regarded as demonstrative.

The point about which there is no question is that in none of the figures of these pupae is there a Y chromosome visible. BRIDGES had found that the Y chromosome, more than any other, tended to stand out clearly. It was usually well isolated and remained condensed so that it stained deeply and sharply. The difference in length of the limbs was clearly defined and enabled him to distinguish the J-shaped Y from the equal-armed second and third chromosome V's.

The spermatogonial groups are of greatest value but are very rare. Probably a half-dozen were examined of which three or four were fairly clear. Two (3 and 4) in one pupa were demonstrative. In the spermatogonial groups there was an unpaired rod,—the X,—two pairs of V's, and a pair of spherical chromosomes. These two XO figures should be compared with XY figures from normal males, of which an example is given in figure 2 (a previously unpublished figure furnished by BRIDGES).

There were no satisfactory first-spermatocyte figures, but there were many good "interkineses" (plate 1, figures 5–14) and many "second" divisions (plate 1, figures 15–19) and some spermatid cells (figures 20–21). Interkinesis groups were expected to be of two types equally numerous: one type possessing an X (figures 5–14) was frequent and clear; the other possessing no X (figures 18, 19) was infrequent and uncertain.

The second-spermatocyte divisions were likewise expected to be of two types. In one type (plate 1, figures 15, 16, 17) there is the normal 4-chromosome group, including an X, approaching each pole. This type was clear and unmistakable. In the other type there are only three chromosomes,—two V's and the spherule,—with no X chromosome, but in none of the few cases observed is it certain that an X has not been lost in sectioning.

The spermatids were likewise expected to be of two types corresponding to the products of the second divisions. The X type was clear, but again the type in which two V's and a spherule are present without a sex-chromosome could not be clearly demonstrated.

To sum up, the no-X type of interkinesis group, the no-X type of second-division and the no-X type of spermatids that would be expected to result from reduction divisions in an XO male seemed not to be as numerous as the corresponding X type. Indeed, there is some question

as to whether any were really present. Furthermore the number of nuclei in the "interkinesis" stage was exceptionally large; and no certain first-maturation divisions were found. Also it was observed that in the "interkinesis" often the small chromosomes were not in contact with each other as they should be if they were sister chromosomes about to undergo an equational separation. These findings may mean that one division is suppressed and that in the stage labeled "interkinesis" the V's attached by their apices represent unreduced autosome pairs while the double-length rod is the X with an equational split. The single maturation division following this stage would give each of the two resulting spermatids the full haploid number of chromosomes.

There are two general points in connection with the figures that should be mentioned. BRIDGES (1916) had observed that in the gonial figures of males and females there was a striking demonstration that the forces which caused the splitting of a chromosome into two daughter chromosomes and that responsible for the drawing of those daughters to the poles were independent. The metaphase chromosomes begin to split and separate at the free outer ends and the separation travels progressively inward until the chromosomes are in contact at the apex only of the V's and at the inner end only of the X. These daughter parts seem to repel each other and to stand out so that an autosome presents two V's with their apices joined and the free limbs directed poleward. Afterwards the union between the apices is dissolved and the apices travel toward the poles so that the relative positions of the daughter V's are reversed. This repulsion is seen in the clearest fashion in the "interkinesis" figures where the X chromosome opens out at the ends to form a V and the large autosomes become double V's with their free arms directed to the four angles of a tetrahedron, as is clearly shown in plate 1, figure 11 and others.

GYNANDROMORPH "51"

A gynandromorph appeared (July 18, 1915) in culture 51, which was a cross between an eosin miniature female and a wild male. The abdomen was female in size, shape and coloration, and appeared to contain a pair of ovaries. The genitalia were likewise female. The right wing was wild-type (female) and the right half of the thorax was large (female). The left wing was minature and the left side of the thorax was smaller (male) in consequence of which the abdomen was twisted to the left. The bristles also were smaller on the left side of the thorax. The division of the thorax into male (left) and female (right) sides did not extend

LEGEND FOR PLATE 1

The figures in this plate were drawn at table level; tube length 160 mm; Zeiss compensating ocular 12X; and Zeiss apochromatic 1.5 mm oil immersion objective, N. A. 1.30. The figures were then enlarged $2\frac{1}{4}$ diameters, and in reproduction were reduced in the ratio 3:2. The resulting magnification is 5,115 diameters.

Figure 1 (furnished by BRIDGES) is an oögonial plate of a wild female. Two X chromosomes are present as rods. This figure is from a freshly hatched mature fly; the rest of the figures are from pupae.

Figure 2 (previously unpublished figure furnished by BRIDGES) is a spermatogonial plate of a wild male. The Y chromosome has its characteristic unequal-armed J shape. This figure shows a late metaphase group in which the chromosomes are already split; the same split condition is seen in 3 and 4.

Figures 3 and 4 show late-metaphase spermatogonial groups from an exceptional (XO) male. Both are from one individual. In these groups there is an unpaired rod, the X, two pairs of autosomal V's already split, and a pair of spherules, the "fourth" chromosomes.

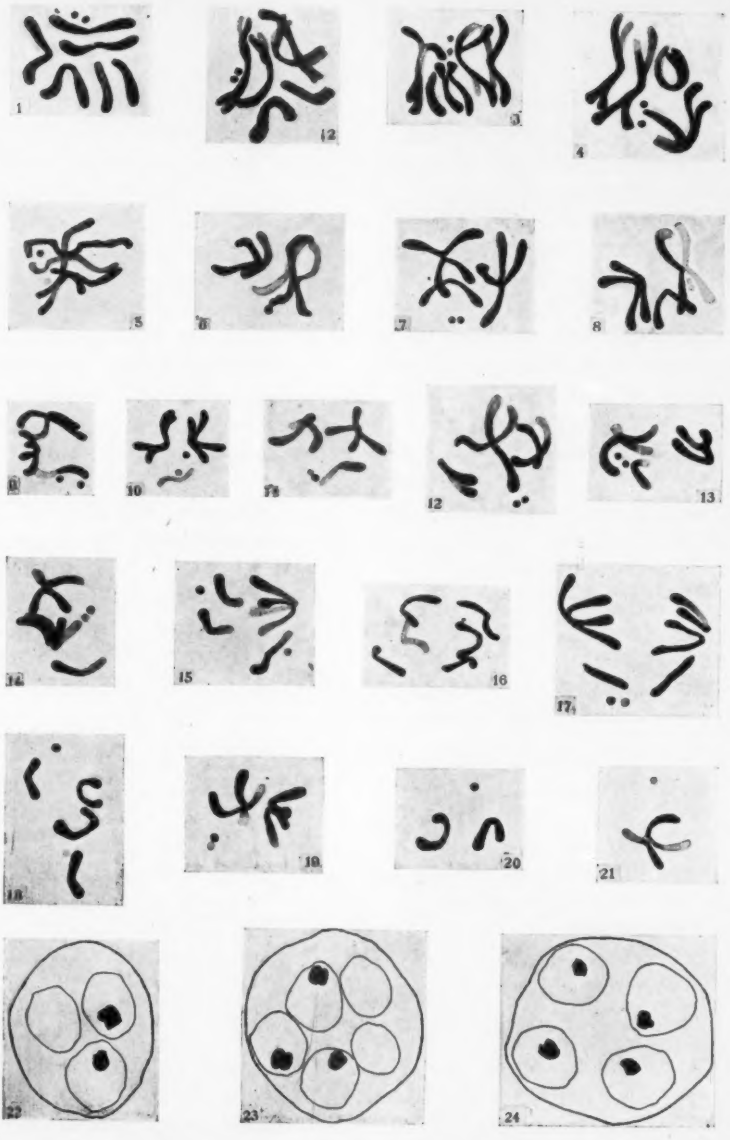
Figures 5–14 from XO males show a few of many good "interkinesis" groups. In "interkinesis" the X chromosome, ordinarily rod-like, splits into a V which opens out into a double-length rod. Likewise the autosomal V's resolve into two V's which remain attached at their apices with the free limbs directed toward the corners of a tetrahedron.

Figures 15, 16, 17, 18, 19 show "second-spermatocyte" division groups. Figures 15, 16 and 17 contain an X, while 18 and 19, appear to be of the other expected type in which there is no X. The nuclei of 18 and 19, may be cut and incomplete, so that no great weight is to be attached to them. There were many "interkinesis" groups, "second-spermatocyte" divisions, and some spermatid groups showing an X present. None showed a Y. Several might well be without an X but none of these latter were conclusive.

Figures 20 and 21 are polar views of two spermatid groups that also appear to be of the no-X type. Both these divisions were cut through the mid-region between the groups, and while no X was detected it can not be certain that no X was present.

Figures 22–24 show giant multinucleated cells. These represent final spermatogonial cells and spermatocytes that failed to form separate cell boundaries; or more probably they have resulted from the coalescence of separate cells in the same stage of development.

In general, the most important point about the figures examined is that in none of them is there a Y chromosome visible. BRIDGES had found that the Y chromosome, more than any other, tended to stand out clearly, was usually well isolated, and remained condensed so that it stained deeply and sharply; this is seen especially well in figure 2. There can be no mistake about the absence of such a Y chromosome from the groups of these slides.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through to the ventral side for both fore-legs bore sex combs and were therefore purely male. The head was small and both eyes were eosin of the male color. The head was therefore entirely male.

MORGAN and BRIDGES (1919) have shown that the usual cause of gynandromorphism in Drosophila is the "elimination" (at an early cleavage division) of one of the two X chromosomes of a female. The parts that descend from the cell that receives only one X become the male parts of the gynandromorph, and the other cells that retain the original two chromosomes give rise to the female parts. An egg bearing an eosin miniature X was fertilized by a sperm bearing a wild-type X. The zygote was of the same type as the regular daughters expected from the cross. But at an early cleavage division (probably the second in this case) one of the two X's that come from the division of the paternal wild-type X, failed to be included in the daughter nucleus, probably being entangled at the mid-plate of the division. This cell received a full complement of autosomes but only one X chromosome—the maternal eosin miniature X. All the parts that developed from this cell (the head, the forelegs, and half the thorax with its wing) were male and were at the same time eosin and miniature. The remaining parts were female (XX) and showed only wild-type characters, since the eosin and miniature carried by the maternal X are both recessive, if both gonads arise from a single isolated cell they should always be either male or female, in this case female.

SOMATIC MUTATION FROM EOSIN TO WILD-TYPE

In a pure stock culture of eosin miniature there appeared (August, 1915) a single male whose left eye was completely red and whose right eye was eosin with a vertical bar of red across the middle. This appearance of red in a pure stock of eosin means a reverse mutation from eosin to the wild type or to an allelomorph not distinguished from the wild type. The peculiar distribution of the red indicates that the mutation occurred in the early embryonic stage in the somatic tissue. This fly was tested by breeding and behaved as does a pure eosin miniature.

BUFF, A SIXTH MUTANT ALLELOMORPH OF WHITE EYE COLOR

In cultures No. 55 and 188 of the eosin miniature series of tests there appeared, (July 28, 1915), besides the expected classes, two and eight females which had long wings like their wild-type sisters but whose eye color, instead of being red, was lighter than that of standard eosin

females and only a little darker than that of eosin males. These light-eyed females were put into a culture bottle where they produced offspring, having evidently been fertilized by their eosin miniature brothers. Unfortunately little attention was paid to the offspring of these females. Apparently half of the males were eosin and half lighter than eosin having a pale cream or "buff" eye color. Most of the buffs were not miniature but a buff miniature was selected for breeding in order to take advantage of the miniature in placing the new mutant.

The buff miniature male was out-crossed to a wild female and gave only red daughters (195) and sons (187) which shows buff to be recessive. About half the F_2 males, but none of the F_2 females, showed the buff color which is thus shown to be due to a sex-linked gene. There was 35.2 percent of crossing over between buff and miniature which suggested that the locus of buff was at least very close to that of eosin. Not one of the sons was eosin in color, which showed that buff was probably not eosin plus a diluter or specific modifier. In subsequent experiments (SAFIR 1916) a total of 14,811 flies involving the linkage of buff and miniature was raised. The crossover value was 35.5 and no eosin appeared. In back-cross experiments involving yellow, buff, and miniature, 7,537 flies were raised which gave a position for buff 1.9 units to the right of yellow. The early yellow white data gave 1.1 as the distance of white or eosin to the right of yellow, but later and better data show that the distance is probably nearer 1.6 which agrees well with the 1.9 here obtained for yellow buff.

Buff males were out-crossed to white, eosin, and cherry females and in each case the F_1 females were not red but were intermediate in color between the parental colors. The formation of compounds together with the practical identity of linkage relations between buff and white shows that buff is an allelomorph of white.

The buff female is of the same intensity of color as the male, as is the case of all the white allelomorphs except eosin.

The white allelomorph series is wild-type, white, eosin, cherry, blood tinged, and buff.

It is apparent that the original light-colored females were buff-eosin compounds and that the buff mutation had occurred in the wild-type father.

GIANT OR MULTINUCLEATED CELLS IN THE TESTES OF THE XO MALES

It had been found that the testes of XO males contained relatively few packs of sperm as compared with a normal male. The reason for this

became apparent when the sections of the testes were examined. The spermatogonial cells were approximately normal in numbers but the final spermatogonial cells and spermatocytes quite frequently failed to form separate cell boundaries.

A rounded syncytium was thus formed that contained two, four, or eight nuclei (plate 1, figures 22, 23, 24). It seems probable that in some cases the entire contents of the small cysts were included within a common cytoplasmic body. It was observed that the nuclei of many of these giant cells seemed abnormal or degenerating. It is believed that these giant cells often die and disintegrate without the formation of spermatozoa. In other cases it seems probable that these giant cells have formed the bundles of non-motile non-separable spermatozoa to be found in the lumen of the testis.

According to the account of WODSEDALEK (1916) such giant spermatogonia and spermatocytes exist in the mule, and their degeneration precludes the formation of spermatozoa and leads to the sterility of the mule.

SUMMARY

In *Drosophila melanogaster*, as in other animals, in normal reduction divisions of XX females the two X chromosomes disjoin from each other and pass to egg and to polar body, respectively, so that each mature egg has one X chromosome.

In his work on non-disjunction BRIDGES found that:

(1) Occasionally the two X chromosomes do not disjoin (primary non-disjunction) but are both retained in the egg (XX egg) or are both extruded to the polar body (zero or no-X egg).

(2) The fertilization of the XX egg by an X sperm gives a non-viable XXX individual. An XX egg fertilizer by a Y sperm gives an XXY matroclinous "exceptional" daughter, which in turn gives "exceptions" due to the action of the supernumerary Y (secondary non-disjunction).

(3) The fertilization of a no-X egg by a Y sperm gives a non-viable zygote (YO). A no-X or zero egg fertilized by an X sperm gives an XO individual which is a patroclinous "exceptional" son.

My further work on primary non-disjunction has led to the following results:

1. The constitution of primary exceptional sons has been proved to be XO by direct cytological examination. Their spermatogonia were found to contain an unpaired X chromosome. No Y chromosome was present. The products of the first, or reductional, divisions were expected to be

nuclei containing an X and nuclei free from an X. The latter type was certainly not present. Other aberrations also were present that suggest that the one maturation division was wholly or frequently suppressed.

2. XO males are indistinguishable in sex-linked characters, in somatic characters, in genitalia, and in mating behavior from XY males, but are absolutely sterile (about 69 tested, including about 15 tested by BRIDGES).

3. The spermathecae and oviducts of females contained no sperm after copulation with XO males.

4. In the testes of XO males were found only a few bundles of sperm and these bundles did not readily break up into individual spermatozoa.

5. The spermatozoa were non-motile.

6. A cytological examination of the testes showed that there were present multinucleated giant cells; the cytoplasm of many cysts consisted of a common rounded mass with no cell division corresponding to the nuclear divisions.

7. The non-motility of the sperm and the compactness of the bundles seem to be a result of the syncytial nature of the cysts.

8. The percentage of primary exceptions observed by BRIDGES was 0.06 (12 in a total of 20,484). The percentage of primary exceptions produced by eosin minature females was 0.18 (87 in a total of 47,545); by vermilion females the percentage was 0.08 (19 in a total of 22,988); by white females, 0.07 (15 in a total of 21,773).

9. The above exceptions were proved to be primary by three tests: (a) The number per culture was too low to be due to secondary non-disjunction; (b) exceptional males were usually tested and all tested were found to be sterile and therefore XO in constitution; (c) sets of eight regular females were tested from each culture giving exceptions and these were proved to be free from Y chromosomes.

10. The sets of tests of daughters gave in the case of the eosin miniature line 48,190 additional offspring of which 18, or 0.04 percent, were primary exceptions. The vermilion-series daughter tests furnished 19,042 offspring of which 12, or 0.06 percent, were primary exceptions.

11. The percentage of exceptions produced by the eosin miniature strain (0.18 on the basis of 47,545 flies) was approximately 3 times as high as in other experiments which all gave nearly the same percentages and totaled 64 in 111,993 flies, or 0.06 percent. It is probable that the eosin miniature strain contained a mutant gene that favors the production of primary exceptions. This gene would be a recessive since the normal percentage of primary exceptions was given by the tested daughters, which were the F_1 from an out-cross of that line.

12. The primary exceptions reported by BRIDGES were 5 females and 7 males. In my tests (and in other cases by BRIDGES) there is a marked excess of males, viz., in the eosin miniature direct tests 17 females and 70 males, in the vermilion tests 2 females and 17 males, in the white tests 2 females and 13 males, in the daughter tests respectively 4 females to 14 males, and 2 females to 10 males. In all (including BRIDGES's) there have been 32 females to 131 males, or a ratio of 1:4.

13. The probable explanation of this excess of male primary exceptions is that they have resulted from a type of "elimination" at the reduction division. The two X chromosomes failed to disjoin from each other, and lagging upon the center of the spindle were caught by the formation of the mid-body, and were prevented from becoming part of either nucleus. All eggs preceded by such non-disjunctional elimination would be no-X and would produce only XO males.

In conclusion, I wish to acknowledge my indebtedness to Prof T. H. MORGAN for his interest in the progress of my work and for his many valuable suggestions, and especially to Dr. C. B. BRIDGES for his interest in supervising the experiments and in the writing of this paper.

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GENETIC STUDIES ON *DROSOPHILA SIMULANS*. I. INTRODUCTION. HYBRIDS WITH *DROSOPHILA MELANOGASTER*[1]

A. H. STURTEVANT

Columbia University, New York City

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INTRODUCTION

Many attempts have been made to hybridize different species of Drosophila, but hitherto all but the combination to be described here have been unsuccessful. The present case is itself only to be considered a partial success, since the hybrids have so far all been completely sterile. Furthermore, since the two parent species are extremely similar and probably have identical chromosome groups, the data that are to be obtained from the study do not throw as much light as might be wished on many of the problems concerning the nature of the specific differences found in the genus Drosophila. Nevertheless, the investigation has led to interesting results bearing on such subjects as interspecific sterility, parallel mutations, chromosome maps, and sex determination.

DESCRIPTION OF *Drosophila simulans*

Drosophila simulans STURTEVANT (1919) is very similar to the well-known *D. melanogaster* Meigen (*ampelophila* Loew), and has been confused with it until recently. There is only one satisfactory character by which

[1] Contribution from the CARNEGIE INSTITUTION OF WASHINGTON.

the two species may always be separated; viz., the external male genitalia (figures 1 and 2). In living or relaxed pinned material the posterior process (P) of the genital tergite appears like a clam-shell in *D. simulans*, and like a small hook in *D. melanogaster*. The eggs (figures 3 and 4) are also characteristically different, but are more variable than the male genitalia, besides being less convenient to obtain. In addition to these characters, the cheeks are a little narrower and the eyes a little larger in

FIGURE 1.—External male genitalia of *Drosophila simulans*. From a specimen cleared and mounted in balsam. A, anal plate; C, clasper; G, first genital tergite; P, posterior process of genital tergite; VI, VII, abdominal spiracles on fifth abdominal tergite.

simulans, and in general the newly hatched specimens have a more definite dark streak down the dorsal surface of the thorax (mesonotum) than has *melanogaster*. With a little experience one can usually separate most of the individuals in a mixed stock by the use of these characters. In addition there are other still more indefinite differences, that are observable when large series are compared. *Drosophila simulans* averages somewhat stouter in abdominal shape and darker in color than *melano-*

gaster. In mixed laboratory cultures *simulans* is apt to be smaller, though in pure cultures or in wild material there is no appreciable size difference.

Though experience will give one the impression that the two forms are distinct and should be classed as "good species," they are so close

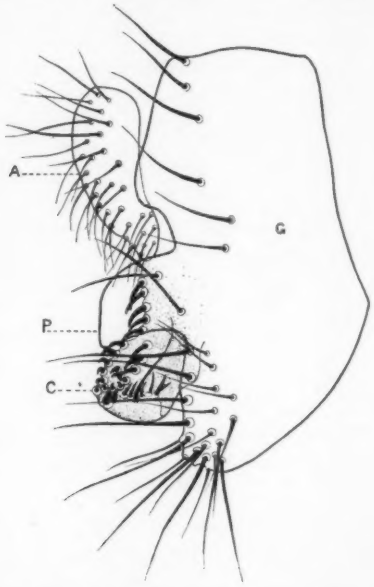

FIGURE 2.—External male genitalia of *Drosophila melanogaster*. Abbreviations as in Figure 1.

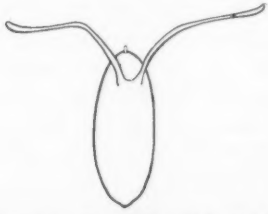

FIGURE 3.—Egg of *Drosophila simulans*.

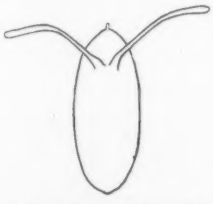

FIGURE 4.—Egg of *Drosophila melanogaster*.

that a technical description of *D. melanogaster* or a drawing of the style familiar to geneticists would pass equally well for *D. simulans*. So far as I have been able to discover, the mating and breeding habits and the length of the life-cycle are identical. Dr. C. W. METZ reports, after a preliminary study, that the chromosome groups are probably also identi-

cal. I am unable to distinguish the larvae, the pupae, or the female genitalia. The structure of the male genitalia and the sterility of the hybrids remain as the only valid grounds for making a specific distinction; but I think there can be no question that these grounds are sufficient.

DISTRIBUTION OF *Drosophila simulans*

I described *D. simulans* from stock collected at Lakeland, Florida. I have also seen specimens from New Hampshire, New York, Minnesota, Virginia, Georgia, Alabama, Costa Rica, Panama, and the Brazilian states São Paulo and Matto Grosso. Rather extensive collections from the Pacific coast and from the West Indies have so far not been found to contain any *simulans*, and it has not appeared in the Old World material that I have seen. *Drosophila melanogaster*, on the other hand, is evidently a cosmopolitan species. I have seen undoubted specimens, determined by examination of male genitalia, from American localities scattered from Nova Scotia to Oregon, California, Florida, Porto Rico, Costa Rica, Chile, and Brazil; and from Holland, Spain, New Zealand, Australia, and Hawaii. It is also recorded from Africa, India, and elsewhere.

Evidence has been presented by JOHNSON (1913) that indicates that *D. melanogaster* was introduced into this country from the tropics, and became common soon after 1870. The arguments advanced by JOHNSON are equally applicable to *simulans*, so far as they indicate the rarity or absence of the species before 1870, since the two forms have practically identical habits, and are so similar in appearance that even when JOHNSON wrote they had never been distinguished. The period at which *simulans* was introduced remains doubtful. It seems certain that QUACKENBUSH (1910) had specimens of it, derived from a stock collected at Woods Hole, Massachusetts, in 1908, and this is the earliest record known to me. The earliest specimen that I have actually seen was collected in Florida in 1912. It is quite probable, however, that an examination of large series of earlier material would show the species to have been established much earlier. The present distribution, outlined above, indicates that the species must have come from South or Central America.

DISCOVERY OF *Drosophila simulans*

At present *Drosophila simulans* and *D. melanogaster* are about equally common around New York City, in Florida, in Alabama, and in southwestern Brazil. Since the two are distinguishable only with some practice, and since a fresh wild stock usually contains both, it seems very surprising

that *simulans* was not accidentally discovered long ago in the course of genetic experiments with *melanogaster*. It must have been in the laboratory many times, but seems to have led to unexpected results only two or three times. The curious "unisexual broods" recorded by QUACKENBUSH (1910) were surely hybrid broods. Professor E. A. ANDREWS reported in correspondence some years ago that he had had difficulty in crossing a mutant race of *melanogaster* to a wild race collected at Baltimore, Md., and it now seems probable that the wild race was *simulans*. Finally, Mr. A. M. BROWN, in the COLUMBIA laboratory, obtained anomalous results with a wild race collected by me in Alabama, which ultimately led to the recognition of *simulans*. Mr. BROWN found that males of this race (now known definitely to have been *simulans*), when crossed to mutant races of *melanogaster*, gave only sterile female offspring.

Because of these results observed by Mr. BROWN, I asked Dr. C. W. METZ to send me a number of wild races from Lakeland, Florida, in the spring of 1919. These were tested by crossing to *melanogaster*, and about half of them were found to give unisexual broods. It was noticed that stocks that gave this result looked somewhat different from ordinary *melanogaster*, and had different eggs. Later, Dr. C. B. BRIDGES discovered the striking difference in the male genitalia. Not until many experiments had been made with the Florida race was it noticed that *simulans* also occurs in many other places. I have since shown by crosses, both to the Florida race and to *melanogaster*, that several New York races and one each from New Hampshire and from Minnesota, behave in the same way as does the Florida race. There can now be no question that the male genitalia, by which these later races were first identified, are a certain index as to the genetic behavior.

I have kept mixed stocks of the two species for several months, using the ordinary laboratory technique for keeping stocks of *melanogaster*, and have found that both species may persist together for at least five months. But in such mixed stocks *melanogaster* is usually far more numerous, and the few individuals of *simulans* are almost always small and pale. When the culture becomes old and dry *melanogaster* seems to be unfavorably affected more quickly, so that the percentage of *simulans* rises. Greater resistance to such unfavorable conditions may perhaps account for the fact that *simulans* maintains itself in nature in spite of the fact that, under favorable conditions, it is outdone by *melanogaster*. In the small numbers and small size of the *simulans* specimens from mixed stocks we apparently have the reasons why it was not discovered sooner, for when one selects flies for mating he usually picks out large vigorous

ones, and such a procedure would almost always lead to the selection of *melanogaster*. Furthermore, even if *simulans* was occasionally selected it would usually fail to cross, and the absence of offspring would simply be ascribed to sterility.

Drosophila melanogaster ♀ × *D. simulans* ♂

This cross was first made by QUACKENBUSH (1910), to judge from his account. It was also made by Mr. A. M. BROWN, as stated above. I first obtained it in May, 1919, and have since repeated it many times. If the mother is an ordinary XX individual the result is that *only female offspring are produced*. Hundreds of offspring have been obtained from matings of this type, without the production of males.[2] These hybrid females are completely sterile (with *simulans*, *melanogaster*, *obscura*, or hybrid males) and have rudimentary gonads, as QUACKENBUSH first showed.

These females always have the sex-linked characters expected of females from the cross. If the mother carries recessive sex-linked genes that are not present in the father, the corresponding recessive characters are never present in the hybrid daughters. This is true for all the recessive mutant characters tested, 12 in number. If sex-linked recessives are present in the *simulans* father, these also do not appear in the hybrid females. Three such have been tested. It follows that the hybrid females have two X chromosomes, and are not transformed males.

It has been shown by BRIDGES (1916) that females of *melanogaster* that have a Y chromosome (XXY) produce exceptional offspring of two types, due to the production of XX and Y eggs, respectively. The first (XX) type of eggs, if fertilized by Y sperm, produce daughters that get all their sex-linked genes from their mother, and are therefore matroclinous exceptions to the usual behavior of sex-linked characters. The second (Y) type of eggs, if fertilized by X sperm, give rise to sons that get their sex-linked genes from their father, and are thus patroclinous exceptions to the usual behavior of sex-linked characters. In order to further analyze the sex-ratio of the hybrid families, I have crossed XXY *melanogaster* females by *simulans* males. The result has been that *only the regular daughters and the exceptional sons are produced*. The other two classes, regular sons and exceptional daughters, are entirely absent.

[2] The results to be presented below indicate than an occasional male, due to primary non-disjunction, is to be expected. One male obtained in the cross involving scarlet *simulans* (to be described in part III of these studies) may have been of this nature.

Two experiments of this type have given the same result, and have produced a total of 128 regular females and 59 exceptional males.[3]

Hybrid males produced in this way are sterile, and have rudimentary testes. They are in no way distinguishable from the hybrid males obtained from the reciprocal mating. They have the sex-linked characters of the father, and are identified as non-disjunctional exceptions by this fact.

Drosophila simulans ♀ × *D. melanogaster* ♂

The reciprocal hybrids are more difficult to obtain. They have so far been produced most often from yellow mothers, except in one mating made by QUACKENBUSH. This mating has been made most often by Prof. T. H. MORGAN (1920), but I have made it successfully a few times. Usually the *only offspring produced are regular sons*, but MORGAN records having sometimes obtained also a few regular daughters, especially as the cultures became old. I have on one occasion used *simulans* females from a stock known to carry extra Y's, and obtained a hybrid female that was undoubtedly a non-disjunctional exception.[4]

As in the case of the reciprocal mating, all these hybrids were completely sterile.

ANALYSIS OF RESULTS

The results of the four types of interspecific matings may be tabulated as follows:

melanogaster ♀ (XX) × *simulans* ♂ = regular ♀♀.
melanogaster ♀ (XXY) × *simulans* ♂ = regular ♀♀ + exceptional ♂♂.
simulans ♀ (XX) × *melanogaster* ♂ = regular ♂♂ + a few regular ♀♀.
simulans ♀ (XXY) × *melanogaster* ♂ = regular ♂♂ + exceptional ♀♀.

Regular females will probably occur in the last mating in small numbers, as in the third mating. It is possible that other classes may appear, but none of them, aside from the exceptional males of the last mating, can survive in nearly as large a percentage of cases as do the types noted.

Analysis of these results indicates that the egg cytoplasm must play a part in determining what types survive. We may represent this cytoplasm by the symbol C, and denote the origin of it and of the chromo-

[3] The large number of exceptions is due to the fact that a strain of "high non-disjunction" was used, and does not indicate a higher viability of the exceptional males.

[4] The nature of this female was certain because she had the characteristic appearance of a hybrid, and because she was known to have received a *melanogaster* third chromosome but not a *melanogaster* X chromosome.

somes by sub-letters (m for *melanogaster*, s for *simulans*). The types investigated may then be tabulated as follows:[5]

♀	♂
$C_mX_mX_s$,—lives	$C_mX_mY_s$,—dies
$C_mX_mX_sY_m$,—lives	$C_mX_mY_mY_s$,—dies
$C_mX_mX_mY_s$,—dies	$C_mX_sY_m$,—lives
$C_sX_mX_s$.—usually dies	$C_sX_sY_m$,—lives
$C_sX_mX_sY_s$,—usually dies	
$C_sX_sX_sY_m$,—lives	

The Y would not be expected to affect the result, and may probably be disregarded. The above formulae then simplify as follows:

♀	♂
$C_mX_mX_s$,—lives	C_mX_m —dies
$C_mX_mX_m$,—dies	C_mX_s,—lives
$C_sX_mX_s$,—usually dies	C_sX_s,—lives
$C_sX_sX_s$,—lives	

This table presents many anomalous points. The difference between the two kinds of regular females (C_m X_mX_s and C_s X_mX_s) can only be due to a difference in the cytoplasm of the mother, since the chromosomes of these two types must be identical.[6]

That hybrid females should develop in *melanogaster* cytoplasm only in case they carry a *simulans* X is surprising. This result must be due in part to the autosomes (or to the *simulans* Y), since C_m X_mX_m, that dies as a hybrid, is the formula of the normal *melanogaster* female. In general, it appears that hybrids develop only if they carry a *simulans* X, but that in the presence of *simulans* cytoplasm a *melanogaster* X usually inhibits development even though a *simulans* X is also present.

FATE OF C_mX_m MALES

It has been assumed in the preceding discussion that the missing classes of hybrids die. It has been shown, by a consideration of the distribution of the sex-linked characters, that they are not changed over into any of the other classes. The possibility of selective fertilization is the only remaining alternative to the assumption that the individuals belonging to the missing classes are formed but die before they reach the adult stage. The evidence now to be presented shows that in the hybrid

[5] The autosomes are alike in all cases (one member of each pair from each parent) and have therefore been left out of the formulae.

[6] It is, of course, probable that the nature of this egg cytoplasm is determined in the first instance by the chromosomes of the female in which the eggs develop.

cultures from *melanogaster* ♀ × *simulans* ♂, many individuals die in the larval stage; and there is every reason to believe that these represent the missing males.

An eosin vermilion forked *melanogaster* female was mated to a forked *simulans* male. Larvae were observed in about five days, and the pair was removed to a fresh vial. A small amount of food was supplied, and the eggs laid on it were counted and removed, individually, to fresh food. Forty-seven eggs collected in this way gave rise to 14 adults, all of them hybrid forked females as expected. An attempt was made to count the pupa-cases after the adults had all emerged, but this was not found possible. It was clear from the attempt, however, that most of the pupae gave rise to adults, since only a few unhatched puparia could be discovered. Evidently, then, there is heavy mortality in the egg or larval stage. Observation of the isolated eggs indicated that larvae hatched from most of them. A few days after hatching it was noticeable that the larvae were of two sizes;—one type seemed to have grown normally, while the other was much smaller. It had previously been observed that a few larvae were usually present in a hybrid culture at the time adults ceased emerging, and that a number of hybrid larvae died without pupating. These observations indicate that the male larvae grow slowly and never pupate. Their history has not yet been followed in detail, nor has the fate of the other missing classes of hybrids been determined.

SEX-RATIOS OF OTHER SPECIES HYBRIDS

Numerous examples of anomalous sex-ratios among species hybrids are on record. These are evidently due to different causes, as will appear from an examination of a few examples.

When the domestic cow is fertilized by the male bison, the male offspring have a hump, derived from the bison, which is so large that it usually prevents their being born alive. The female hybrids lack this hump, and are born normally (BOYD 1914, etc.). Here, then, an abnormal sex-ratio is brought about at the time of birth by causes external to the hybrid itself,—i.e., by the structure of the mother.

GUYER (1909) has collected data on species hybrids among certain birds, and has shown that there is a decided excess of males in F_1. Although it is possible that some of the individuals were in reality females that had developed male plumage as a result of imperfect development of their ovaries, the evidence indicates a real excess of males. RIDDLE (1916) has recorded the reverse relation (excess of females) for the cross

Streptopelia risoria × *S. alba* (species of doves) under certain conditions. The cross involves a sex-linked gene, and should give only dark males and white females. Examination of RIDDLE's data for the sex and color of the hybrids shows that, contrary to RIDDLE's own conclusion, the excess of females is not due to "changing over" of males. The female hybrids failed to receive a maternal Z chromosome and would therefore be expected to be females. The data also show that there is not a high enough mortality of embryos to account for the deficiency of males. The only remaining alternative explanation is that, under the conditions of the experiment, the Z chromosome went to a polar body at reduction oftener than it remained in the egg.

GOLDSCHMIDT (1916, 1917, 1919), HARRISON (1919), and others working with Lepidopterous hybrids have often obtained broods that were largely or entirely of one sex. HARRISON has shown that in at least one instance there is not a high enough mortality to account for the results; and both authors have presented evidence indicating strongly that the results are due to a "changing over" of the sex of half the individuals. Crosses in the same groups often produce intersexes, that are more or less intermediate between males and females. These intersexes commonly replace one of the expected sexes, while the other is present in the expected proportions. Intersexuality may be very slight or more and more marked. The unisexual broods evidently represent the final stage where one sex has been completely changed over into the other. The nature of this change is not yet satisfactorily explained; but it is clear that the anomalous sex-ratios are due to causes different from those operating in RIDDLE's doves or in the cow-bison hybrids. The intersexes will be discussed again, in connection with the *simulans* intersexes (part III of these studies).

The *melanogaster-simulans* hybrid sex-ratio evidently represents still a fourth type. It is not due to a changing over of sex, as shown by the distribution of the sex-linked genes. It is not due to a preferential maturation of the egg, since the female Drosophila is not heterozygous for sex. It is not due to external mechanical difficulties, since both types of larvae emerge from the eggs. The cause is an internal one, but acts merely by killing certain classes of larvae.

DESCRIPTION OF HYBRIDS

In general, *melanogaster-simulans* hybrids, of all types that have been observed, are intermediate between the parent species in those characters in which the parents differ. The male genitalia are shown in figure 5.

The width of the cheeks is also intermediate, as is the streak in the dorsal surface of the thorax. Since the hybrid females do not produce eggs, their egg-filaments cannot be examined.

In several respects the hybrids differ from both parents. The complete sterility of both sexes has already been referred to. It is connected with the rudimentary condition of the gonads. The ovaries especially are very minute, and in the testes no sperm have been seen. The hybrids

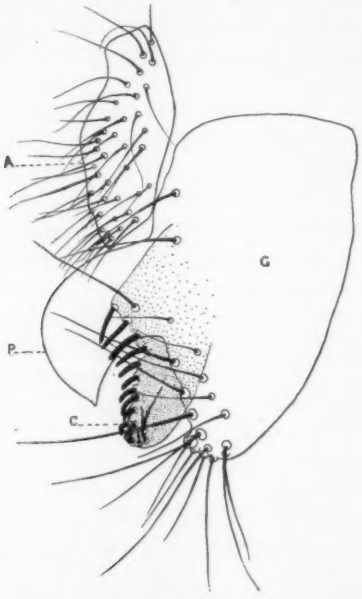

FIGURE 5.—External male genitalia of hybrid male. A, anal plate; C, clasper; G, first genital tergite; P, posterior process of the genital tergite. The upper part of the first genital tergite was broken off in this specimen; this part of the plate is normally shaped as in the two parent species.

(more especially the females) frequently have fewer dorso-central and scutellar bristles than the number (4 of each sort) that is normal for each parent species. All of these bristles are sometimes missing, though in both pure species individuals with extra bristles are more frequent than those with bristles missing, and I have never seen wild specimens in which more than two were missing. Other bristles on the thorax (noto-pleurals, supra-alars, post-alars) are also often missing in the hybrids but not in the pure species. Another peculiarity that is not infrequent among the

hybrids (though less common than the missing bristles) is a broken or missing cross-vein. In the same category belongs abnormally banded abdomen. Both of these characters are very rare in wild individuals of each parent species. None of these characteristics of the hybrids suggests any related species of fly known to me.

These characters that appear in the hybrids but not in either parent species must be due to complemental genes carried by the two species. Since they are evidently dominant genes, it may be surmised that F_2, were it possible to obtain it, would include individuals with numerous other new characters, due to recessive complementary genes.

SEXUAL SELECTION

A small series of observations indicates strongly that females of *melanogaster* or of *simulans* are far more likely to mate with males of their own species than with those of the other species. This is probably the reason why so many cross-matings fail to produce offspring. Both kinds of males will court females of either species, apparently indiscriminately; and that it is the females that are responsible for the small percentage of successful cross-matings is also indicated by the fact that normal *simulans* females have seldom been successfully crossed to *melanogaster* males. QUACKENBUSH used a female that had been subjected to heat, and nearly all later crosses have been from yellow females. I have shown (STURTEVANT 1915) that yellow females of *melanogaster* are more easily mated with by *melanogaster* males than are wild-type females.

Sexual selection, then, is one means whereby the two species are kept from crossing. It would, however, probably be ineffective if fertile hybrids were produced when cross-mating does occur.

Hybrids, of both sexes, have been seen to mate with both parent species. No sufficient data are at hand to show what preferences, if any, are shown by the hybrids, or how readily the parent species will mate with them.

SUMMARY

1. *Drosophila simulans* is a species closely similar to *D. melanogaster* in structure, habits, and chromosome group.

2. The two species may be crossed, though many cross-matings fail, apparently because females do not readily allow males of the other species to mate with them.

3. *Drosophila melanogaster* ♀ × *D. simulans* ♂ produces only female offspring, unless the mother is XXY in constitution. In the latter case

non-disjunction occurs, and regular daughters and exceptional sons are produced.

4. *Drosophila simulans* ♀ × *D. melanogaster* ♂ usually produces only male offspring, but females sometimes come in small numbers. One exceptional female, due to non-disjunction, has also appeared.

5. The regular females from these two matings are of the same chromosome constitution, but in one case they live and in the other they usually fail to emerge. This relation can only be accounted for by supposing that the egg cytoplasm influences the result.

6. Hybrids survive only if they carry a *simulans* X. In the presence of *simulans* egg cytoplasm and a *melanogaster* X, survival is not usual even if a *simulans* X is also present.

7. In the cross of *melanogaster* ♀ × *simulans* ♂, evidence is presented indicating that the regular males die in the larval stage.

8. The sex of the hybrids corresponds in all cases to their chromosome constitution. XX is female, X is male.

9. Hybrids of all classes are completely sterile, and have rudimentary gonads.

10. The hybrids are intermediate between the parent species in all respects in which the latter differ.

11. The hybrids often show some characters (absence of certain bristles, missing or broken cross-vein, abnormally banded abdomen) not present in either pure species.

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